JOURNAL OF AGRICULTURAL RESEARCH

VOL. 48 WASHINGTON, D.C., APRIL 1, 1934 NO. 7

RELATION OF LENGTH OF DAY TO GROWTH OF TIMOTHY [1]

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INTRODUCTION

Since 1920, when Garner and Allard [2] first announced their discovery of the principle of photoperiodism, many investigators have published results of studies of this phenomenon as it applies to many kinds of plants.[3]

In some plants, earliness and lateness are not the only characteristics that are affected by changes in the length of day. It has been shown that in timothy (*Phleum pratense* L.) not only time of blooming but also elongation of the internodes of the stems and the number of elongated internodes per culm are affected by the length of day.[4]

In a preliminary test conducted in 1930 it was found that timothy plants that bloom and mature early when grown under natural conditions respond to days artificially made of uniform lengths in a different way from plants that are later in their development. This discovery suggested the desirability of conducting an experiment for the purpose of determining the responses of timothy plants that bloom and mature at different times to days that range in length by uniform gradations from relatively short to relatively long.

When plants propagated vegetatively from a single original timothy plant are grown at stations in different latitudes, it has been found that in the Northern Hemisphere the season for blooming of these plants does not progress from south to north at the uniform rate per day of one quarter of a degree, as predicted in Hopkins' bioclimatic law.[5] On the contrary, the plants bloom relatively later at the southern stations and relatively earlier at the northern stations than this law prescribes.[6] One possible explanation for this is that the development of the plants in the South during early spring is delayed by the relatively short days that occur in southern latitudes, and in northern latitudes the development is hastened by the relatively long days that occur during late spring and early summer.

[1] Received for publication Nov. 17, 1933; issued June 1934.

[2] GARNER, W. W., and ALLARD, H. A. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Research 18: 553-606, illus. 1920.

[3] LUBIMENKO, V. N., and SZEGLOVA, O. A. L'ADAPTION PHOTOPÉRIODIQUE DES PLANTES. Rev. Gén. Bot. 40: [577]-590, [675]-689, [747]-768, illus. 1928.

[4] EVANS, M. W. THE LIFE HISTORY OF TIMOTHY. U.S. Dept. Agr. Bull. 1450, 56 pp., illus. 1927.

[5] HOPKINS, A. D. PERIODICAL EVENTS AND NATURAL LAWS AS GUIDES TO AGRICULTURAL RESEARCH AND PRACTICE. Monthly Weather Rev. Sup. 9: 5-42, illus. 1918.

[6] EVANS, M. W. RELATION OF LATITUDE TO TIME OF BLOOMING OF TIMOTHY. Ecology 12: 182-187, illus. 1931.

MATERIAL AND METHODS

For this investigation selected timothy plants were used. These plants are referred to as strains of timothy. All the strains listed in tables 2 and 3, except the last four, were selected from plants grown from seed of American origin; the last four originated from seed from northern Europe and are later than plants ordinarily occurring in meadows in the United States. These strains have been arranged in a series, according to the dates when the first florets bloomed on plants growing with natural illumination at Washington, D.C., in 1931. They represent uniform gradations from the earliest to the latest. The experiment was conducted at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D.C.

On December 16, 1930, each of several plants was divided, and each subdivision, bearing the same number that had been assigned the original plant, was placed in a 3-inch pot. On January 26, 1931, each plant was transferred to a 4-inch pot. The plants were grown in a cool greenhouse (50° to 55° F.) until February 18, 1931, when they were removed to coldframes built upon trucks on tracks. These trucks were moved out of doors for 10 hours each day until the final tests under the various lengths of day began; during the remainder of the time they were kept in dark houses.

After the experiment was begun, plants of each strain were grown under all or part of the following numbers of hours of illumination each day: 10, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, and 18. In addition, one or more plants of each strain, used as controls, were grown under the natural lengths of day.

The length from sunrise to sunset of the longest day occurring at Washington is 14 hours and 54 minutes. The plants grown with uniform lengths of day less than this maximum were placed in metal cans or buckets on trucks, which were moved into ventilated dark houses for that part of each day during which the plants received no illumination. The plants grown with 15 hours or more of illumination each day were placed out of doors, where the length of day was extended as desired by means of electric lights placed over the plants.

The plants that received 14.5 hours or less of illumination were out of doors each day for the periods indicated in table 1, from the date the test began until the final records were obtained.

TABLE 1.—*Time during which timothy plants receiving illumination for 10 to 14.5 hours daily were out of doors*

Date when test began	Length of period of illumination each day	Hours during which plants were out of doors	Date when test began	Length of period of illumination each day	Hours during which plants were out of doors
	Hours			*Hours*	
Apr. 11	10	From 6 a.m. to 4 p.m.	May 18	13.5	From 5 a.m. to 6:30 p.m.
Do	12	From 6 a.m. to 6 p.m.	Do	14	From 5 a.m. to 7 p.m.
Do	12.5	From 6 a.m. to 6:30 p.m.	Do	14.5	From 5 a.m. to 7:30 p.m.
Do	13	From 5 a.m. to 6 p.m.			

Artificial light was required in order to obtain constant illumination for 15, 16, 17, and 18 hours each day, periods representing approximately the maximum lengths of day for the latitudes 41°, 48°, 53°, and 57°, respectively. This added illumination was furnished for

each group of plants by four 200-watt gas-filled tungsten lights with reflectors, built according to standard specifications. These lights were placed at each corner of a square of such dimensions that the distance from center to center of the lights was 3 feet. The lights, which were kept about 2 feet above each group of plants, were arranged on an adjustable frame so that they could readily be raised as the plants increased in height. Each set of plants was screened by partitions high enough to cut off the direct rays of its group of lights from all the other sets of plants.

In order to maintain illumination for a uniform period each day, it was necessary to use electric lights for different lengths of time. Constantly decreasing daily periods of artificial light were required up to the midsummer solstice, and constantly increasing periods of light after the solstice. Four electric time switches were used to control the required decrements or increments of artificial light, and the necessary changes were made each day. The tests under all four lengths of day, from 15 to 18 hours, began April 11. The lights were turned on at 5 p.m., and were kept on for different periods, so that the different groups of plants were illuminated for 15, 16, 17, and 18 hours, respectively, from sunrise each day.

EXPERIMENTAL RESULTS

EFFECTS OF DIFFERENT LENGTHS OF DAY ON DIFFERENT PHASES OF GROWTH

Insofar as vegetative growth is concerned, the plants thrived under all lengths of day within the range of this experiment. There were, however, great variations (1) in the time when the heads emerged from the enclosing leaf sheaths, (2) in the time of occurrence of the flowering process, (3) in the characteristics of the stems, and (4) in the development of the stems.

Time When Heads Emerge from Enclosing Leaf Sheaths

The date of emergence of the earliest head of each plant from within the enclosing leaf sheath is shown in table 2.

In general, the later in the season the heads appeared on plants grown with natural illumination, the greater was the number of hours of illumination required for the development of the inflorescences on plants of the same strain grown with artificial light. Plants of the 2 strains that were earliest under natural conditions produced inflorescences when subjected to only 10 hours of light each day. Plants of 1 of the 4 strains that were latest under natural illumination required day lengths of 14.5 hours, while none of the plants of the other 3 strains of this group produced inflorescences under less than 15 hours of illumination daily. Plants of the other strains had a minimum light requirement of between 10 and 14.5 hours each day for the development of inflorescences; in general, the minimum number of hours of illumination required tended to increase gradually as the time of heading became later on the plants of these strains when grown under natural conditions.

As the number of hours of illumination each day increased, the date of heading on the plants of any strain gradually became earlier until the optimum day length had been attained; after this, even with continued increase in the length of day, the date of heading remained practically the same. This statement may be illustrated by the

TABLE 2.—*Dates in 1931 when the first heads appeared on plants of different strains of timothy, grown with natural daylight from 10 hours to full length of day each day and with added artificial light to obtain illumination of 15 to 18 hours daily, near Washington, D.C.*[a]

Strain no.	Date of emergence[b] of first heads in plants grown with indicated hours of illumination											
	Full day	10	12	12.5	13	13.5	14	14.5	15	16	17	18
19456	May 20	June 22	May 18	May 18	May 20	(c)	(c)	(c)	(c)	(c)	(c)	(c)
19457	May 25	June 23	June 5	June 3	June 3	May 22	(c)	(c)	(c)	(c)	(c)	(c)
19458	May 26	----------	----------	June 15	June 16	(c)	(c)	(c)	(c)	(c)	(c)	(c)
15092	{May 28 / May 26}	----------	June 27 / June 24	June 8 / June 10	June 15 / ...do....	May 28 / ...do....	May 28 / May 26	May 28 / May 25	{May 18	May 15	May 13	May 13
11902	June 1	----------	July 24	July 1	June 25	June 3	June 1	June 1	May 20	May 18	May 14	May 18
6127	{June 8 / June 10 / June 12}	----------	----------	----------	----------	{June 18 / June 27}	{June 15	June 8	May 25	May 20	May 19	...do....
6743	{June 8 / June 6}	{July 20	----------	June 26	June 27	June 11	June 8	June 4	...do....	June 1	...do....	May 15
11966	{June 13 / June 16}	----------	----------	----------	July 22	July 3 / June 23	June 22 / June 15	June 17 / June 13	{May 29	May 21	...do....	May 16
9220	June 18	{----------	Aug. 3 / July 31	July 21 / July 11	Aug. 3 / July 13	July 7 / July 13	June 26 / June 30	{June 19	June 1	May 19	May 18	May 18
12421	June 19	----------	----------	----------	----------	July 14	...do....	June 22	June 5	May 25	May 20	May 21
15485	{June 25 / June 26 / June 27 / June 30}	----------	----------	----------	----------	July 22	July 9 / July 10	June 30 / June 27	June 8 / ...do....	May 26 / ...do....	May 22 / May 21	May 22 / May 21
19416	{July 3 / July 7}	----------	----------	----------	----------	----------	----------	----------	June 20	June 1	May 26	May 26
15445	{July 3 / July 6 / July 11 / ...do....}	----------	----------	----------	----------	----------	----------	----------	June 13	May 29	May 23	May 21
19459	{July 15 / July 13 / July 17}	----------	----------	----------	----------	----------	----------	July 20	June 16 / June 18	June 1 / June 3	...do.... / May 25	May 23 / May 25
19460												
19461												

[a] To obtain daily exposures of 10 to 14.5 hours required keeping the plants in a darkened house for a certain number of hours each day; to obtain daily exposures of 15 to 18 hours required the use of artificial light to supplement the normal length of day.

[b] Leaders indicate that no heads emerged under the period of illumination specified; more than 1 date of emergence or line of leaders merely indicates that the test included 2 or more sets of plants.

[c] No plants were grown under this period of illumination.

records of the plants of strain no. 9220 (table 2). Under natural conditions, the first head appeared on the control plant on June 18. On the plant grown with 10 hours of light each day, no inflorescence developed; on that grown with 12 hours of light the tip of the first head appeared on July 31. With increasing length of day the time of heading gradually became earlier, until with 16 hours of illumination the time of heading occurred on May 19. When exposed to 17 and 18 hours of light each day, the heads of the plants appeared on May 18, practically the same date as on the plant grown under a 16-hour day.

Time of Flowering

The relative dates at which the first florets appeared on plants of the different strains grown with different periods of illumination corresponded, in a general way, to the relative dates at which the first heads had appeared on the same plants about 12 to 15 days earlier.

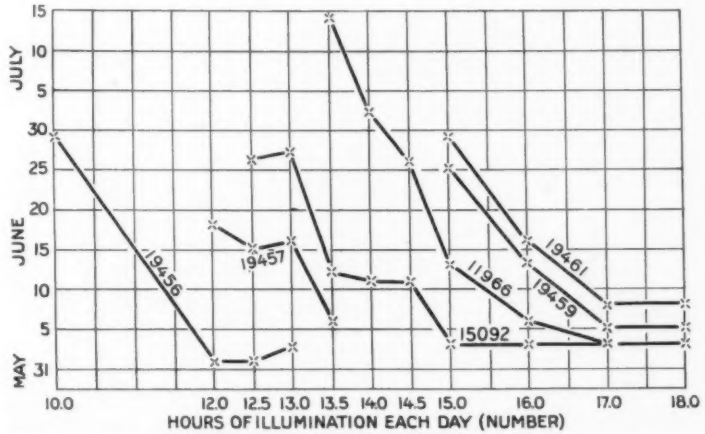

FIGURE 1.—Dates in 1931 when the first florets bloomed on different timothy strains (indicated by numbers), grown under various constant daily periods of illumination.

In plants that under natural conditions range gradually from very early to very late the response to days of different lengths, as indicated by the time of flowering, is quite consistent (table 3). On the control plant of strain no. 19456, the first florets bloomed on June 3; this was the earliest plant to bloom under natural conditions. On the plant of this strain grown with 10 hours of light each day, the first florets bloomed on June 29. As the date of flowering became later on plants of the different strains grown under natural conditions, the minimum number of hours of illumination under which any florets bloomed gradually increased, until, in the case of strain no. 19461, which was the latest to bloom under natural illumination, no florets appeared on plants grown under days of uniform length of less than 15 hours.

As the length of day increased, the dates on which the florets on the plants of any particular strain bloomed gradually became earlier up to the optimum period of illumination, after which there was no further increase in earliness. This tendency is shown by the records in table 3 and the curves in figure 1. Under natural conditions, the first florets

TABLE 3.—*Dates in 1931 when the first florets opened on plants of different strains of timothy, grown with natural daylight from 10 hours to full daylight each day and with added artificial light to obtain illumination of 15 to 18 hours daily, near Washington, D.C.*[a]

Strain no.	Date of first flowering [b] of plants grown with indicated hours of illumination											
	Full day	10	12	12.5	13	13.5	14	14.5	15	16	17	18
19456	June 3	June 29	June 1	June 1	June 3	(c)	(c)	(c)	(c)	(c)	(c)	(c)
19457	June 8		June 18	June 15	June 16	June 6	(c)	(c)	(c)	(c)	(c)	(c)
19458	June 11			June 27	June 26	(c)	(c)	(c)	(c)	(c)	(c)	(c)
15092	June 12 / June 13		July 14	June 22 / June 26	June 27 / June 29	June 12 / do	June 11 / June 13	June 11 / June 12	June 3	June 3	June 3	June 3
11902	June 17 / June 18		Aug. 4	July 13	July 10	June 19	June 16	June 18	June 6	do	do	Do.
6127	June 19 / June 23					June 29 / July 6	June 26	June 22	June 9	June 5	do	Do.
6743	June 22 / June 23				July 17	June 25	June 29	June 19	June 12		do	Do.
11966	June 26 / June 30					July 14 / July 20	July 2 / July 6	June 26 / June 29	June 13	June 6	do	Do.
9220	June 29 / July 2			July 18	Aug. 1	July 23	do / July 13	July 2	June 16	June 5	do	Do.
12421	June 30						do	July 3	June 15	June 8	June 5	June 4
15485	July 6 / July 7 / July 9						July 20	July 9	June 20	do	do	June 5
19416	July 11							July 11	June 22	do	do	June 4
15445	July 14 / July 20								July 2	June 16	June 12	June 8
19459	July 17 / July 20								June 25	June 13	June 5	June 5
19460	July 22 / July 25							July 29	June 29	June 16	June 8	June 4
19461	do / July 27								do	do	do	June 8

[a] To obtain daily exposures of 10 to 14.5 hours required keeping the plants in a darkened house for a certain number of hours each day; to obtain daily exposures of 15 to 18 hours required the use of artificial light to supplement the normal length of day.

[b] Leaders indicate that no florets opened under the period of illumination specified; more than 1 blooming date or line of leaders merely indicates that the test included 2 or more sets of plants.

[c] No plants were grown under this period of illumination.

on plants of no. 11966 bloomed on June 26. When the plants were grown under days 10 to 13 hours long, no florets bloomed; when they were grown with 13.5 hours of light, florets began to bloom on July 14. As the length of day increased, the date of blooming became gradually earlier up to days of 17 hours, under which the earliest florets bloomed on June 3. When the length of day was increased from 17 to 18 hours, the date of earliest bloom remained the same, June 3. There was a similar response to days of different lengths in the plants of other strains.

The later the plants of any strain of timothy grown under natural conditions produce inflorescences and florets in bloom, the greater is the length of day required for normal development when the plants are grown with days of various constant lengths. This statement is illustrated in figures 2 to 6, which show the plants as they appeared on July 1. The plants of strain no. 19456, the earliest under natural conditions, when grown with 12 hours of light each day produced

FIGURE 2.—Plants of timothy strain no. 19456, grown under different lengths of day, as indicated by number of hours on containers. The control plant (*C*) was grown under natural length of day. Photographed July 1, 1931.

inflorescences on which florets had bloomed before July 1. The plants of strain no. 15092 required a day of about 13.5 hours, and those of strain no. 6127 a day of 14 hours for normal growth. The plants of no. 19461, which was the latest strain used in this experiment, required a day of 16 hours for the production of normal culms with inflorescences.

If the natural length of day is too short for the development of culms and inflorescences of any timothy plant, it may be artificially increased, by means of electric light, to the daily number of hours of illumination which the plant requires. This is illustrated in figure 7, which shows plants of strain no. 19461 grown with natural illumination and with 15, 16, 17, and 18 hours of light each day. On the plants grown with natural illumination no inflorescences appeared until July 13, whereas on the plants grown with 16, 17, and 18 hours of light each day, normal culms and inflorescences had developed before June 25.

FIGURE 3.—Plants of timothy strain no. 15092, grown under different lengths of day, as indicated by number of hours on containers. The control plant (*C*) was grown under natural length of day. Photographed July 1, 1931.

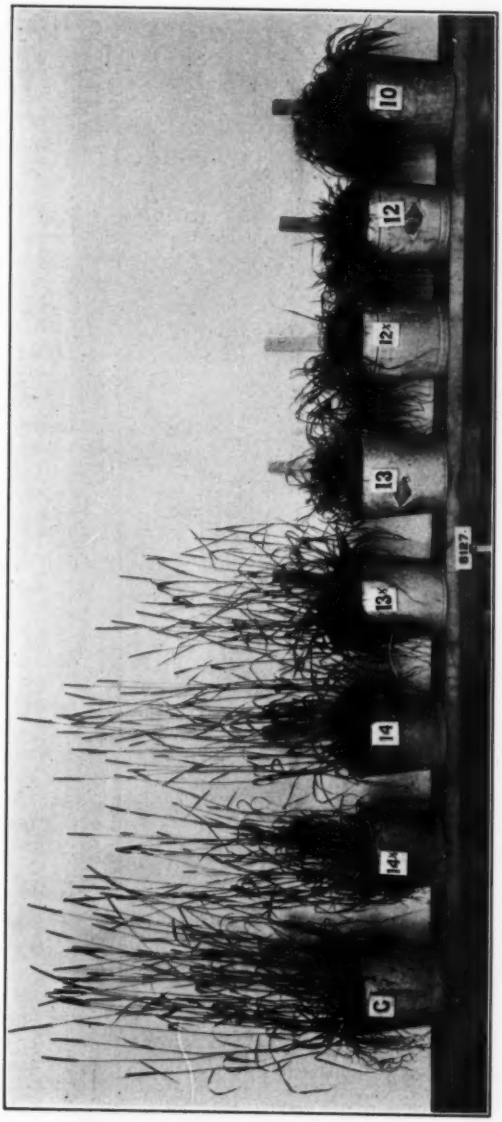

FIGURE 4.—Plants of timothy strain no. 6127, grown under different lengths of day, as indicated by number of hours on containers. The control plant (*C*) was grown under natural length of day. Photographed July 1, 1931.

FIGURE 5.—Plants of timothy strain no. 15485, grown under different lengths of day, as indicated by number of hours on containers. The control plant (*C*) was grown under natural length of day. Photographed July 1, 1931.

Characteristics of Stems

The stems of plants which are grown with sufficiently long periods of illumination each day to enable the inflorescences to develop in a normal manner and on which the florets bloom at a normal time usually grow upright. In this experiment the plants that were grown under days too short for normal development of the inflorescences were commonly characterized by stems which were declined or which were more or less procumbent at the base, sometimes bearing inflorescences with proliferations. Figures 3 and 4 show that the plant of strain no. 15092 grown with 12.5 hours of illumination and the plant of strain no. 6127 grown with 13.5 hours of illumination daily had a tendency to a spreading habit of growth owing to the declined position of the stems.

On plants of all strains except those that were earliest under natural conditions, no elongation of the stems occurred under the shortest periods of illumination. Thus, on the plants of no. 6127 (fig. 4), there was no elongation of stems under 10 to 13 hours of illumination daily; under 13.5 hours of illumination, elongation occurred although the stems were somewhat declined; on the plants grown under 14 and 14.5 hours of light each day and those grown under natural illumination, the stems grew in an upright position.

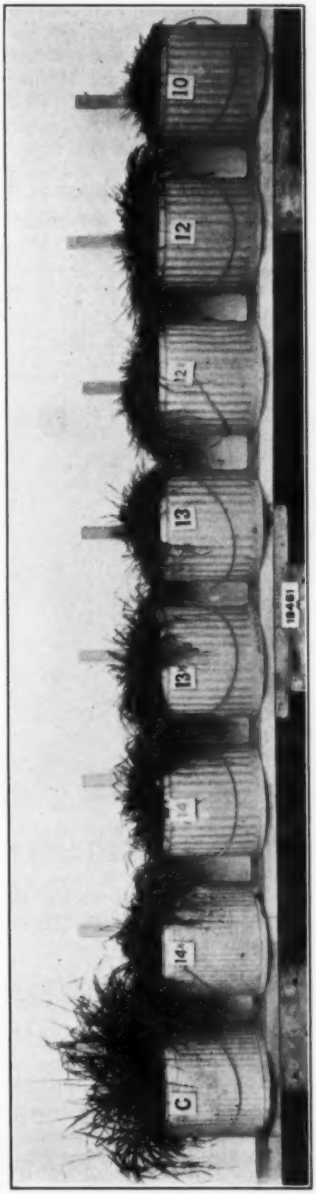

FIGURE 6.—Plants of timothy strain no. 19461, grown under different lengths of day, as indicated by number of hours on containers. The control plant (*C*) was grown under natural length of day. Photographed July 1, 1931.

Length of Stems

At the time of the first blooming of the florets of a timothy plant, the stems on which the inflorescences are borne have attained only a part of their final length.[7]

The data obtained in this experiment show that when the first florets bloomed the actual length of the stems varied according to the number of hours of daily illumination under which the plants had been grown. The records show

[7] EVANS, M. W. Pp. 10-13. (See footnote 4.)

that generally, as the length of day increased, the length of the stems of the plants of any strain on the date when the first florets bloomed gradually increased to the maximum. After this maximum had been reached, the length of the stems was not essentially changed by continued increase in the length of day. For example, when the plants of timothy no. 11902 were grown with 12 hours of light, the longest stem was 16 inches at the time the florets began to bloom; as the length of day increased, the length of stem at the time the first florets bloomed also increased by fairly uniform steps to a maximum length of 41 inches on the plant grown with 15 hours of light each day. On the plants grown with 16, 17, and 18 hours of illumination daily, the length of the longest stem at the time the first florets bloomed was either 40 or 41 inches, practically the same length as on the plant illuminated for 15 hours each day. Table 4 and figure 8

FIGURE 7.—Plants of timothy strain no. 19461 grown under natural length of day (*C*) and with days longer than normal, as indicated by number of hours on containers. This strain of timothy is the latest one (when grown under natural conditions) that was used in this experiment. Photographed June 25, 1931.

show the average length of the longest stems of the plants in groups of early, medium, and late strains of timothy grown under different lengths of day.

RESULTS OF CONTINUED GROWTH UNDER DAYS TOO SHORT FOR DEVELOPMENT OF CULMS AND INFLORESCENCES

In relation to those plants which were grown under days of short lengths and on which only vegetative growth had taken place prior to July 1, the question arises as to whether continued growth for a longer time under the same conditions would have resulted in the development of culms and inflorescences. The results obtained with the plants of strain no. 15485 are fairly typical and may be used to illustrate the general behavior, in this respect, of the plants of all the strains of timothy studied. Figure 9 shows the condition of the plants

of this strain on August 10, 6 weeks after the plants in figure 5 were photographed. On plants upon which partial development of culms occurred before July 1, there was a somewhat more advanced development on August 10. On plants that were grown with 10, 12, 12.5, and 13 hours of light each day, and on which only vegetative growth had taken place prior to July 1, no further development occurred as a result of 6 weeks of added growth. If timothy plants are grown, therefore, under days too short for the formation of culms and inflorescences these will not be formed even though the period of growth under the same light conditions is extended.

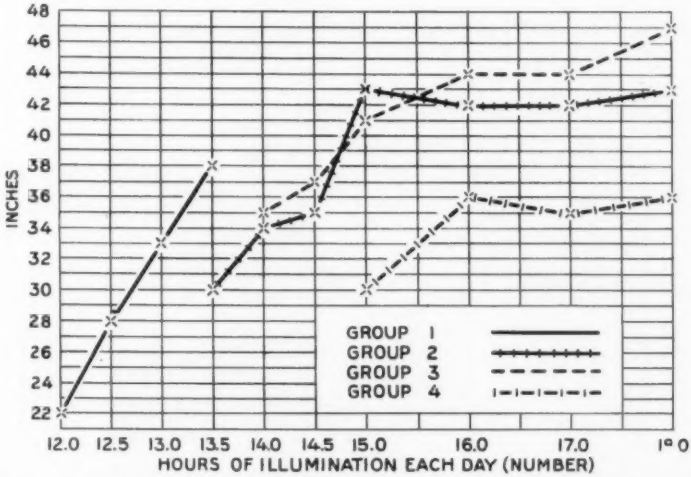

FIGURE 8.—Average length of the longest stem (in inches) of early (group 1), medium (groups 2 and 3) and late (group 4) timothy plants grown under various constant daily periods of illumination.

TABLE 4.—*Average length (inches), on the date when the first florets bloomed, of the longest stem of timothy plants grown under various constant daily periods of illumination and grouped according to relative earliness* [a]

Group	Average length (inches) of the longest stem grown under indicated hours of illumination daily [b]											
	Natural	10	12	12.5	13	13.5	14	14.5	15	16	17	18
1	38	.....	22	28	33	38	(c)	(c)	(c)	(c)	(c)	(c)
2	34	.....	.....	.....	.....	30	34	35	43	42	42	43
3	39	.....	.....	.....	.....	.....	35	37	41	44	44	47
4	27	.....	.....	.....	.....	.....	.....	.....	30	36	35	36

[a] When grown under normal conditions, plants of group 1 (nos. 19457 and 15092) were the earliest strains of timothy used in this experiment; those of group 2 (nos. 11902, 6127, and 11966) were the next earliest, those of group 3 (nos. 9220, 12421, and 15485) were next, and those of group 4 (nos. 15445, 19459, 19460, and 19461) were the latest.

[b] Leaders indicate that no average was available, because flowering either did not take place or some of the strains failed to flower under the indicated period of illumination.

[c] No average given because there were no plants of no. 19457.

FIGURE 9.—Plants of timothy strain no. 15485, grown under different lengths of day, as indicated by number of hours on containers. The control plant (*C*) was grown under natural length of day. Photographed August 10, 1931. Some increase in the length of the stems on plants illuminated for 13.5 hours or more each day since July 1 may be observed by comparing this photograph with figure 5. Under 13 hours or less of light each day, no growth in length of the stems had taken place.

COMPARISON OF EFFECTS OF NATURAL AND ARTIFICIAL ILLUMINATION

The length of the longest day at Rosslyn, Va., where this experiment was conducted, is 14 hours and 54 minutes, or 14.9 hours. Tables 2 and 3 show that in most strains of timothy the heads appeared and the florets bloomed at approximately the same time on plants illuminated for 14.5 hours each day as on the control plants; on the plants grown under days 15 hours long, the heads appeared and the florets bloomed earlier than on the plants grown with natural illumination.

EARLINESS AND LATENESS IN TIMOTHY AN ADJUSTMENT TO DIFFERENT LENGTHS OF DAY OCCURRING AT DIFFERENT TIMES

In timothy, as in some other plants,[8] the earliness or lateness of different strains is evidently a matter of the adjustment of the plants to length of day. In figure 10 the strains of timothy are arranged according to the dates when the first florets bloomed on the plants grown with natural illumination; the first heads had appeared on these plants in practically the same order. Figure 10 also shows the dates on which the first heads appeared and the first florets bloomed on plants of most of these strains when grown with 18 hours of light each day, the maximum length of day employed. When the length of day was increased to 18 hours, plants of those strains that are late under natural conditions produced heads and florets in bloom almost as early as plants of those strains that are early under natural conditions (fig. 10).

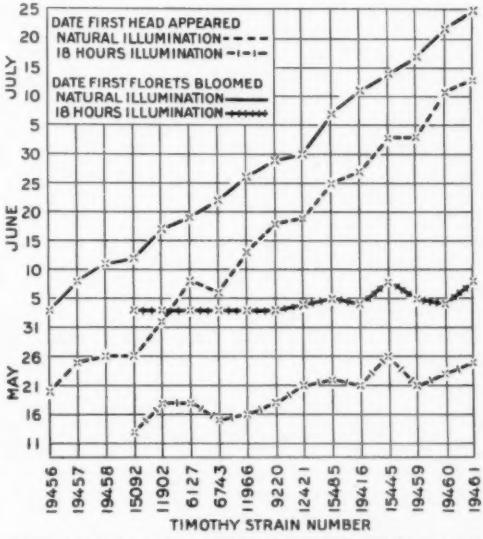

FIGURE 10.—Dates when the first head appeared and the first florets bloomed on timothy plants grown near Washington, D.C., under natural illumination and under 18 hours of illumination each day. Note that plants of those strains that are late under natural conditions in the latitude of Washington, D.C., when grown under 18 hours of daily illumination produced heads and florets in bloom nearly as early as plants of those strains that are early under natural conditions in the same locality.

The results of this experiment indicate that in any locality some timothy plants produce inflorescences and florets in bloom earlier than other plants because they are adapted to relatively short days. On those plants that are adapted to longer days, the appearance of the heads and the flowering process are delayed until the days lengthen.

[8] GARNER, W. W., and ALLARD, H. A. PHOTOPERIODIC RESPONSE OF SOYBEANS IN RELATION TO TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS. Jour. Agr. Research 41: 719–735, illus. 1930.

SUMMARY

At the Arlington Experiment Farm, Rosslyn, Va. (near Washington, D.C.) there were grown under days of different lengths strains of timothy plants that when grown under natural conditions constitute a series ranging by fairly uniform gradations from very early to very late in the time their inflorescences appear, florets bloom, and seeds mature. In addition to the control plants grown under natural illumination, plants were grown with the periods of illumination artificially regulated by means of dark houses and electric lights to days 10, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, and 18 hours long.

Insofar as vegetative growth is concerned, the plants grew well under all lengths of day within the range used in this experiment. There were great variations, however, with respect to the time of emergence of the heads from the enclosing leaf sheaths, the flowering process, and the characteristics and development of the stems.

The later the plants of different strains of timothy produce inflorescences and florets in bloom when grown under natural conditions, the greater is the length of day required for normal development when the plants are grown under days of different uniform lengths.

As the length of day is gradually increased above the minimum under which development of culms with inflorescences occurs, the stems lengthen and the time of the appearance of the inflorescences and the blooming of the florets gradually is shortened up to an optimum length of day. If the length of day is increased above this optimum, there is little added effect—at least up to 18 hours, the greatest length of day under which plants were grown in this experiment.

If the length of day is too short for the development of culms and inflorescences within the time that these processes ordinarily occur, continued growth of the plants for a longer time under the same length of day will not induce their development.

With a day of 14.5 hours, the timothy plants developed at about the same time as plants grown under the natural lengths of days occurring at Washington, D.C., which gradually increase up to a maximum of 14.9 hours at the summer solstice and then gradually decrease. When plants were grown under a day 15 hours long, they developed earlier than the control plants.

In timothy the earliness or lateness of different strains is evidently chiefly a matter of the adjustment of the plants to the lengths of day. The results of this investigation indicate that, in any locality, some timothy plants produce inflorescences and florets earlier than others because they are adapted to relatively short days. On those plants that are adapted to longer days, the appearance of the heads and the flowering process are delayed until, with the advancement of the season, the days increase to the lengths which these plants require for development.

DISTRIBUTION OF OXYGEN AND CARBON DIOXIDE IN MUSHROOM COMPOST HEAPS AS AFFECTING MICROBIAL THERMOGENESIS, ACIDITY, AND MOISTURE THEREIN [1]

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INTRODUCTION

At the present time stable manure is the medium almost universally used for cultivating the common mushroom (*Agaricus campestris* L.). Growers have learned from experience that a period of composting is necessary before the manure is suitable for making up into beds. A rapid fermentation during which comparatively high temperatures are generated is apparently desirable. In general the rate of decomposition of the manure and the suitability of the finished compost for mushroom growing seem to be largely dependent on the size and shape of the compost heap, its height and compactness, the quantity of water added during turning, the thoroughness of the mixing, and the number of days between turnings. These factors probably influence the condition of the finished compost primarily by establishing in the compost heaps conditions of aeration, moisture, and temperature, which in turn establish the trend of the development of the microbial and insect population of the heaps.

The studies described in this paper were undertaken in order to improve composting practice by learning something of the distribution and interaction of these physical and biological factors in typical mushroom compost heaps. At first the writers were concerned principally with recording the temperature, aeration, moisture content, and acidity in all parts of standard mushroom compost heaps. As the observations progressed it became apparent that conditions are radically different in different parts of compost heaps and that the factors of temperature, moisture, and acidity are dependent on aeration, presumably through its effect on microbial activity, in a roughly predictable manner. From these observations an attempt has been made to derive principles that will give the experimenter an approximate conception of the conditions of aeration, temperature, moisture, and acidity to be expected in all parts of stable manure composted as for mushroom culture in heaps of any size or shape.

[1] Received for publication Nov. 28, 1933; issued June, 1934.

METHODS

Ordinary soil thermometers were used for taking temperatures in compost heaps. After the metal tips of these thermometers are heated the instruments may be removed from the compost and read before the mercury begins to fall. For readings at depths greater than 1 foot it was necessary to add extensions to the handles. Complete immersion of these thermometers seems to cause little difference in the readings, and this may be determined and a correction applied. The use of ordinary thermometers of any type is not desirable for three reasons: (1) The fall of the mercury is very rapid once the instruments have been removed from the manure and it is consequently very difficult to take accurate readings; (2) in drawing the thermometers from the bottom of the heaps through the warmer interior the readings change materially; and (3) it is necessary to punch holes in the heaps larger than the diameter of the thermometers in order to drop them in, thus admitting currents of air that frequently change the temperature.

In taking temperatures the thermometers were placed at horizontal and vertical intervals of 6 inches or 1 foot in the heap, depending on the time available and the accuracy desired. After each reading the thermometers were cooled to about 80° F., put into the same holes, and shoved down to the next level. The readings obtained were recorded on crosshatched paper, and the temperature contours were filled in later.

Samples of air from within the compost heaps were obtained by means of a metal tube having a sharply pointed end. Just behind the point four holes were bored for the air to enter. This tube was connected with rubber hose to an Orsat gas-analysis apparatus and could be thrust into the pile at any point for sampling. The air was removed from the tubing before each sample was taken. Samples from within the heap were passed first through a solution of potassium hydroxide to absorb the carbon dioxide and then through alkaline pyrogallol to absorb the oxygen. The probable error of the Orsat apparatus under these conditions seemed to be about 0.2 percent. At times the error of sampling was probably several times this figure. A possible source of error is recognized in the presence within the compost heaps of gases, other than carbon dioxide and oxygen, that might be soluble in potassium hydroxide or alkaline pyrogallol. But in all probability the presence of such gases in small quantities would have little bearing on the problem under consideration and would not affect the evidence or the conclusions in any way.

The hydrogen-ion concentration of samples from different parts of the compost heap was determined with a portable potentiometer by the quinhydrone method. The determinations were made in the field so that the samples were tested only a few minutes after they were removed from the compost heap. There is a tendency for the readings of manure samples to drift toward the alkaline side after the quinhydrone is added. To equalize this effect all samples were allowed an equal period (4 minutes) between the adding of the quinhydrone and the taking of the readings. Preliminary tests showed an average difference of only 0.05 between the pH values of aliquot samples of compost tested with the hydrogen electrode and those tested with the quinhydrone electrode.

TESTS FOR CARBON DIOXIDE AND OXYGEN

Samples of air were taken at first from typical mushroom compost heaps at the Arlington Experiment Farm, Rosslyn, Va., 3 or 4 feet high and without artificial ventilation. Later, aspirations were made from commercial storage heaps 5 to 6 feet high, and from heaps with artificial ventilation at the ground level. In all heaps without artificial ventilation the oxygen content of the air in the interstices of the compost decreased steadily as the bottom center of the heap was approached from the sides or from the top. Anaerobic conditions were usually found within 2 or 3 feet from the side of the heap and 1 foot from the upper surface. Carbon dioxide analyses of the same samples of air indicated a corresponding increase in carbon dioxide as the lower center of the heap was approached. These phenomena are presumably due to the presence of an actively respiring microbial flora.

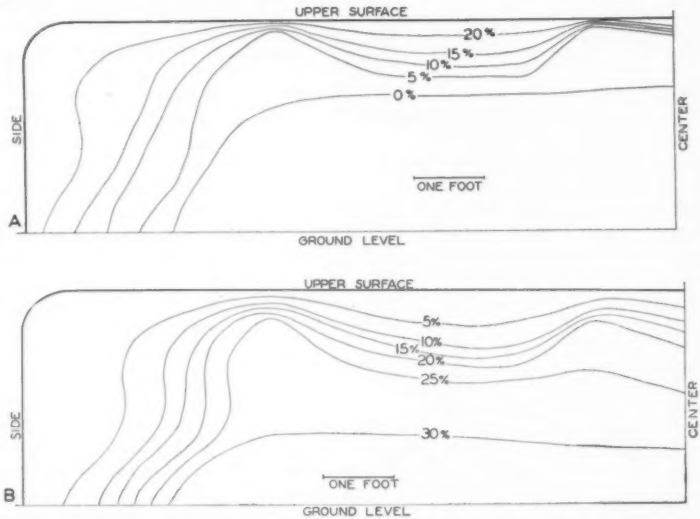

FIGURE 1.—Cross section of a mushroom compost heap from side to center, 12 feet from the end, showing the concentrations of oxygen (*A*) and carbon dioxide (*B*).

Contours showing the concentration of oxygen and carbon dioxide in a typical unventilated heap 3 feet deep are given in figure 1. The dip in the contour lines which appear 5 feet from the side of the heap suggests convection currents.

In most samples in which the oxygen content was more than 1 percent, the sum of the oxygen and carbon dioxide percentages was approximately 21 percent. This, of course, is roughly the percentage of oxygen in the ordinary outside atmosphere, and the constant recurrence of this figure may be taken to indicate that the average respiratory ratio $\left(\frac{cc CO_2}{cc O_2}\right)$ of the heterogeneous microbial population of the compost heap approximates unity under aerobic conditions.

The increase in concentration of carbon dioxide seems to reach a limit at approximately 30 percent. The fact that manure decom-

position is arrested in this section is easily observed while the compost heap is being turned, especially during the second turning. At that time the manure in a mound-shaped region in the lower central part of the heap is distinctly "greener" than the remainder of the heap. No attempt was made to determine whether the 30 percent carbon dioxide content is responsible for this retarded fermentation; nor were analyses made of the remaining 70 percent for combustible gases, such as hydrogen and methane, which are known to be generated under similar conditions.

Tests were made to determine the rapidity with which the concentration of carbon dioxide builds up after the manure is turned. As shown in figure 2, it was found that the carbon dioxide content begins to increase rapidly immediately after turning. In 7 hours the carbon dioxide concentration had reached approximately 20 percent, and no oxygen could be detected.

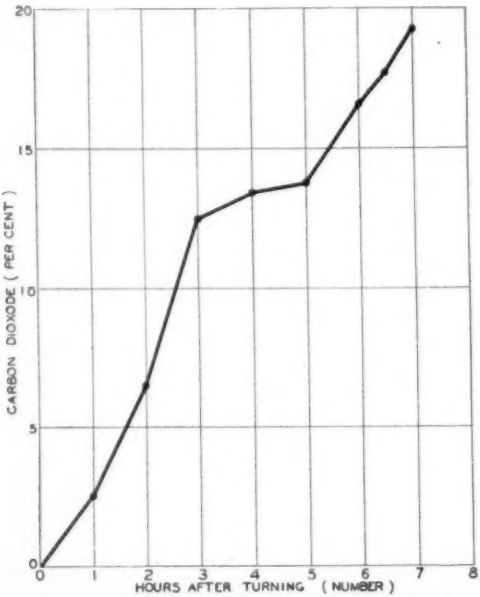

FIGURE 2.—Rate of accumulation of carbon dioxide in center of mushroom compost heap after turning.

For several years a few commercial mushroom growers have been placing ventilating tunnels of lattice work under the center of their compost heaps to speed up decomposition. To determine the effect of ventilation of this kind, bench tile was laid on the ground across an experimental compost heap and aspirations were made at different levels above the tile and at different lateral distances from the tiled area. The results of carbon dioxide analyses from these aspirations are given in figures 3 and 4. It should be noted that there is no anaerobic region in any part of the heap above the ventilation tile. Unlike the conditions in ordinary heaps the percentage of oxygen is higher at the bottom than at 1 or 2 feet from the top. The data in figure 4 indicate that the lateral extension of the effect of ventilation tile on aeration is rather limited. In the heap studied anaerobic conditions prevailed at a lateral distance of 2 feet from the tiled area.

TESTS FOR TEMPERATURE

Two or three days after the compost was mixed the contours of temperatures were found to be substantially the same in all the flat

heaps studied. Typical contours are shown in figures 5 to 8. In general the exterior 3 or 4 inches of the compost heaps varies from slightly above air temperature to above 100° F., depending on the moisture content and the tightness with which the manure is packed. Consistent temperatures begin to be found at a depth of about 6 inches. At the ground level temperatures are relatively low, usually

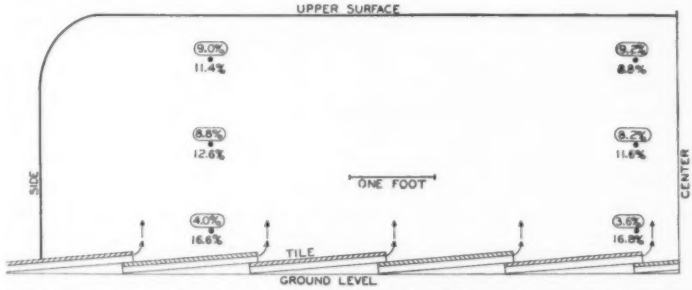

FIGURE 3.—Cross section of one half of compost heap ventilated with greenhouse bench tiles at ground level. Numbers encircled represent concentrations of carbon dioxide; other numbers represent concentrations of oxygen, at points indicated by dots. Arrows indicate currents of air.

from 110° to 120° at points within the heap 2 to 4 feet from the side, then dropping as the center of the heap is approached until temperatures of less than 100° may be encountered. Above the ground there is a similar temperature range. The low-temperature region forms a low mound in the center of the pile roughly corresponding

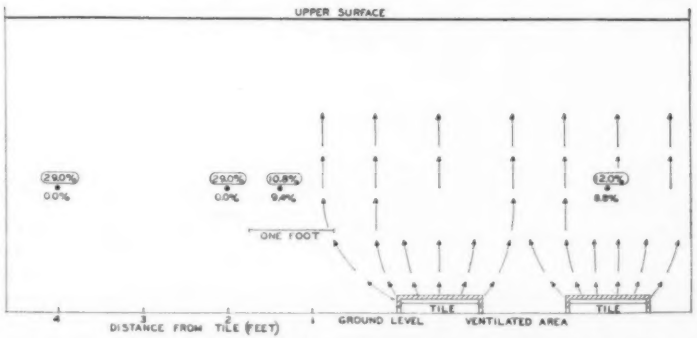

FIGURE 4.—Longitudinal section of portion of compost heap ventilated with greenhouse bench tiles. Section taken across tiles to show percentages of oxygen and carbon dioxide immediately above, and at different lateral distances from source of air. Numbers encircled represent percentages of carbon dioxide; other numbers represent percentages of oxygen, taken at points indicated by dots. Arrows represent currents of air.

to the anaerobic region. Above this the layers of successively higher temperatures rise in more or less regular strata, following the outlines of the mound. The hottest portion of the heap occupies the space between the sides of the heap and the central anaerobic mound. It usually extends from 6 inches to 2 feet down from the top and from 1 to 4 feet in from the sides. Though usually somewhat oval, it

varies in shape and may contain from 2 to 5 square feet in cross section. In this region, forming a ring like a huge elongated doughnut about the center of the pile, the temperature is usually in the neighborhood of 170°, although 182° was recorded on one occasion.

In view of the work of numerous investigators on microbial thermogenesis (*6*, *7*, *8*),[2] there can be little doubt that this distribution

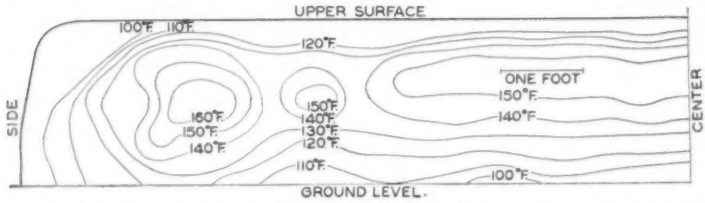

FIGURE 5.—Cross section 6 feet from end of compost heap 2 feet high, showing temperature contours.

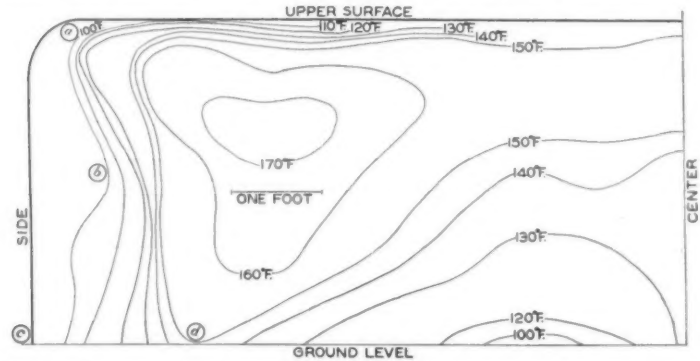

FIGURE 6.—Cross section through compost heap 4 feet high, showing temperature contours. Letters in circles designate points referred to in the text.

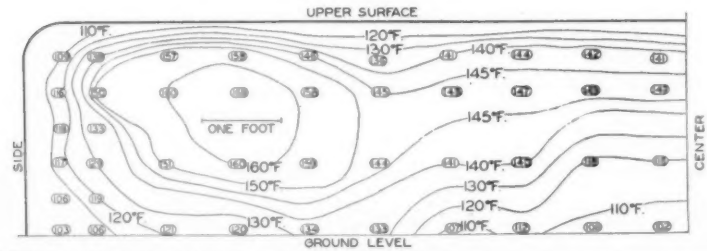

FIGURE 7.—Cross section 6 feet from end of compost heap 3 feet high, showing temperature contours. Ellipses indicate points at which temperatures were taken. Small figures encircled represent temperatures at those points.

of temperature is due largely to the thermogenic activities of an active microbial population.

The warm region apparently is favorable for the accumulation of heat because it is well insulated from outside temperatures and at the same time is comparatively well supplied with oxygen. The outer

[2] Reference is made by number (italic) to Literature Cited, pp. 600 ,601.

layers are cooler because of the lack of insulation from the outside; and the lower central region is cooler because the lack of oxygen retards the microbial activity. The slight extension of the high-temperature region into the adjacent anaerobic region is probably due to the conduction of heat from the aerobic region.

Although moderate aeration seems to be necessary for the production of high temperatures, an excessive current of air through decomposing material may have a cooling effect. This was especially noticeable in heaps of artificial manure made at the Arlington Experiment Farm in the fall of 1930. In these experiments some heaps were made with straw as it came from the straw stack; whereas other heaps were made with the same weight of straw and chemicals, but the straw used had been chopped by means of a corn cutter into 3-inch lengths. There were 12 pairs of these comparable heaps, and in every case during the early part of the composting period the heaps made with short straw were from 40° to 80° F. warmer than those made with long straw. After a few weeks of composting, when the long straw had lost much of its stiffness, the difference was not so noticeable.

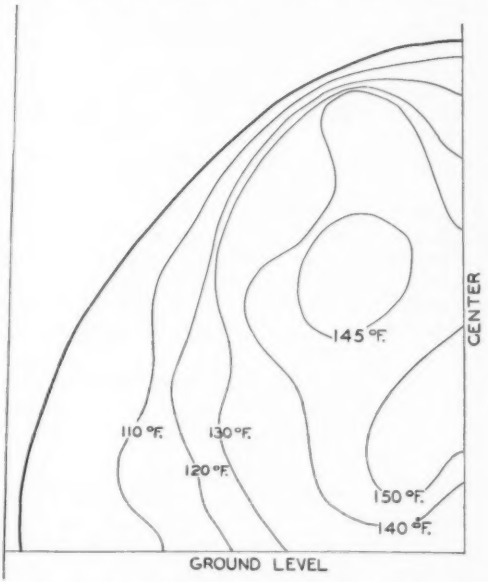

FIGURE 8.—One half of cross section through center of conical compost heap, showing temperature contours.

Irregularities in the exterior contours of the heaps illustrated in figures 5 and 6 may also have been caused by excessive aeration due to convection currents. In hand-turned piles there is usually more or less "flaking", or stratification, on oblique lines from the center, and the air passes into the heap along these strata. This is probably the cause of the convex contour in figure 6, the warm air rising at *a*, drawing in cool air at *c*, and causing the convexity at *b*. The extension of the hotter regions toward *d* is probably due to oxygen brought in by this fresh air, which is available for the heating of the manure beyond the point where the cooling effect of the excessive aeration is felt. In more uniformly turned piles the contours would be expected to be more regular, as in figure 7.

In heaps of the same area, but 2 feet in depth, the contour pattern is practically the same on a vertically compressed scale as in the higher heaps, except that the cool central mound tends to occupy a

slightly greater proportion of the heap than is the case in the higher heaps (fig. 6).

A fairly typical contour pattern for conical heaps is shown in figure 8. In this type of heap the cool central region at ground level is relatively small, but the cooling effect of outside air being drawn in at the lower sides influences a relatively far greater region.

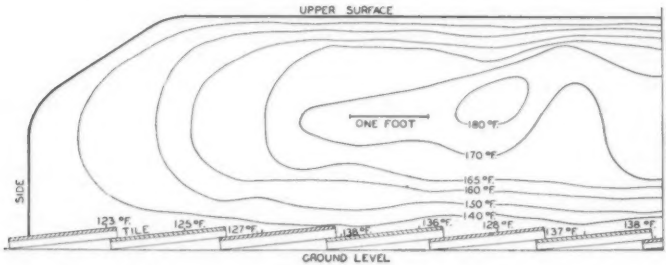

FIGURE 9.—Cross section of compost heap ventilated with greenhouse bench tiles, taken parallel to tiles. Note that whole bottom layer is above 120° F.

In large heaps of the "ridged" type, used in some places, the cross section resembles that of the conical heap, except that the sides are perpendicular to a height of 3 or 4 feet and taper thence to a truncate ridge. These piles are 50 to 60 feet long. In these heaps it would be expected that the contours in cross section would resemble those in figure 8 more or less closely. Because the currents of air have access

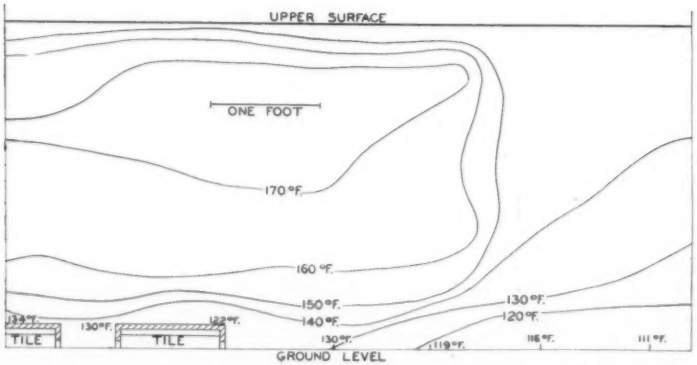

FIGURE 10.—Longitudinal section of compost heap ventilated with greenhouse bench tiles, taken at right angles to tiles.

on only two sides, it is probable that the cooler region at the lower sides will be smaller, and that in the center at ground level larger.

As pointed out in the discussion of aeration, for some time it has been the practice of a few growers to place beneath the composting heaps heavy lattice troughs, or other means of admitting air to the bottom. The effect of this procedure on the temperature contours is

shown in figures 9 and 10. It will be seen that the temperature at the ground level is raised uniformly to above 120° F. In the upper strata the high-temperature areas coalesce, so that the temperature contours are flattened out and the temperature of the whole heap is raised and made more nearly uniform. Undoubtedly this is due to the comparatively uniform distribution of oxygen that was present over the ventilation tiles. When temperatures were taken in a plane at right angles to the tiles, it was found that the parts of the heap more than a foot away from the tiles at ground level were not much affected. As would be expected, a foot or so above the tile the heat of the manure extends laterally for a somewhat greater distance. From the data at hand, however, it would seem that the lateral extension of the effect of aerating devices is rather limited.

In order to ascertain how long it would take compost to attain its maximum heat after turning, with its attendant aeration, the bulbs of recording thermometers were placed in various portions of the 4-foot heaps. In the heaps without tile the temperatures usually reached their maximum in from 18 to 24 hours in the portions of the pile well off the ground but at points at or near ground level continued

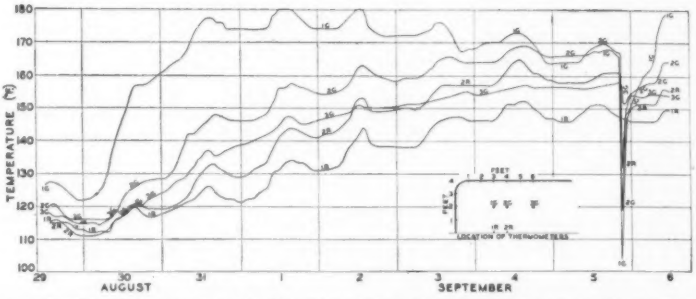

FIGURE 11.—Record of temperatures in different parts of mushroom compost heap after the second turning. The small diagram indicates the positions of recording thermometers by dots and by the symbols 1-G, 2-G, 1-R, 2-R, etc.

climbing for 144 to 168 hours or more, as shown in figure 11. In these piles the difference in temperature between the lowest and highest points at the end of a week or 10 days may be as much as 40° F. and was usually in the neighborhood of 20°. By this time the temperatures are usually running along fairly evenly or dropping slightly. The influence of external weather conditions is very evident. There is a sharp rise in temperature, even at the bottom of the pile, at about noon on warm days, and a corresponding drop at night. Rain causes a sharp drop and a nearly equally sharp rise in temperature, affecting the upper portions of the heaps more than those lower down.

In ventilated compost heaps the temperatures rise more slowly, attaining their maximums in from 48 to 72 hours, the lower portion of the heap being the slowest to warm up. After the maximum temperature has been reached and held for 24 hours or so the temperature slowly drops until the next turning, descending perhaps 25°, as shown in figure 12. The difference between points in the lower and upper portions of the heaps is, as has been pointed out, much less, being only about 8°. The influence of external weather conditions

is seen in these piles but is very much less evident than in the unventilated ones.

HYDROGEN-ION CONCENTRATIONS

There is a considerable difference in the opinion of different workers on the question of the acidity of the compost. Duggar (*4*) in 1905 stated that manure which has undergone fermentation for a few weeks is usually slightly acid in reaction. This statement was accepted for 20 years and substantiated by Bechman (*2*), who found a reaction of pH 6.4 in manure that had fermented 21 days. On

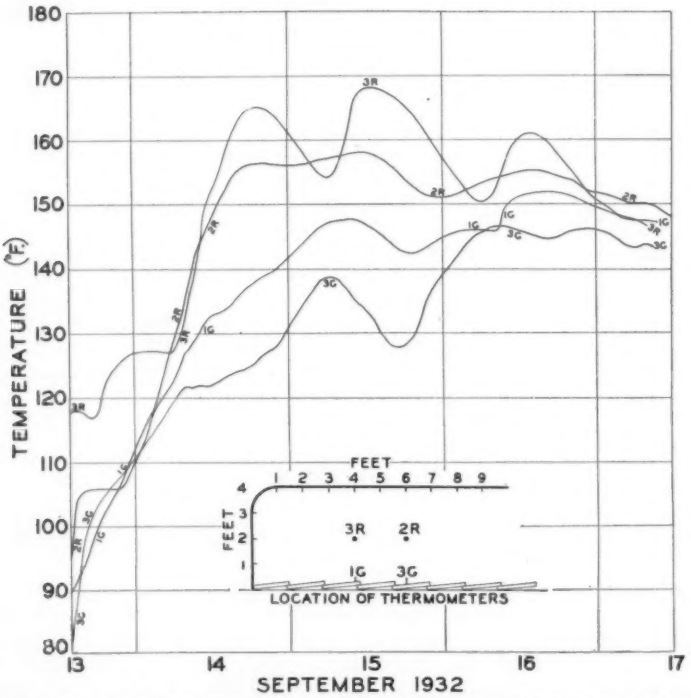

FIGURE 12.—Record of temperatures over tiled portion of mushroom compost heap. The small diagram indicates positions of recording thermometers by dots and by the symbols, 1-G, 3-G, 2-R, and 3-R.

the other hand, Beach (*1*) and Lambert (*9*) found an alkaline reaction in numerous samples of mushroom compost from commercial establishments in eastern Pennsylvania. The pH values recorded in the present study indicate an alkaline condition in well-aerated compost and an acid condition in the parts of the heap composting under anaerobic conditions. It is possible that the discrepancies in the results of different workers can be explained on this basis.

The results of a series of tests of the pH value in different parts of a compost heap are given in figure 13. It is apparent that the outside layer of this heap was largely alkaline or neutral (pH 8.5 to 7.1),

whereas the anaerobic mound at the bottom of the heap was predominantly slightly acid (pH 6.6 to 5.1). The general trend from an alkaline reaction in the outside layers toward a slightly acid reaction in the lower central portion is unmistakable, although there are several notable exceptions. Manure subject to firefang was neutral or alkaline in reaction, and manure over tile ventilation as a rule was more alkaline than manure taken from the bottom of unventilated heaps.

MOISTURE CONTENT

Moisture is one of the most variable factors in a compost heap. In general, mushroom growers attempt to maintain approximately 150 percent of water in the compost on a dry-weight basis. Water is usually added during the process of turning the compost, and in many cases soil is added to the manure to help conserve the moisture. As a result of these practices a moderate moisture content is maintained in most of the compost. On the other hand, there is always a tendency for the compost to dry out excessively on the sides of the heap. This is undoubtedly due to the taking up of moisture by cur-

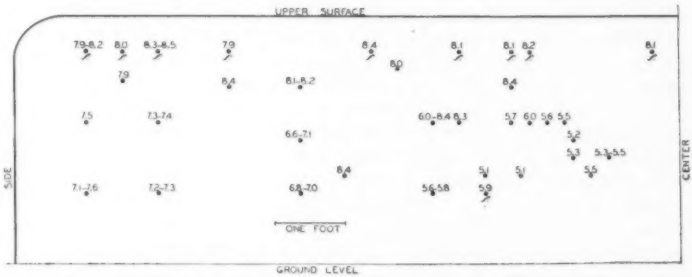

FIGURE 13.—Cross section of compost heap, showing pH values of composting manure, 7 days after second turning, taken at points indicated by dots. The surface layer is alkaline (pH 8.0 to 8.5). The aerated layers on the sides of the heap range from pH 7.1 to 7.6. The wet layer on the bottom center is usually acid (pH 5.1 to 6.6), with occasional alkaline spots (pH 8.0 to 8.4). The firefanged manure (*f*) is aklaline as a rule.

rents of air which are warmed upon entering the heap. A tendency to dry out is noticeable also in the layer of manure in contact with ground-level ventilators. A converse condition is noticeable in the upper 6 inches of the heap. Here the warm air from the lower regions rises to the surface saturated with moisture that condenses when the air reaches the cool outer shell. As a result there is usually a wet layer over the surface of the heap. Toward the end of the composting period there is usually less tendency for the compost to dry out than in the beginning when the straw in the manure is still stiff.

DISCUSSION AND CONCLUSIONS

It is evident from the data presented that there are markedly different conditions of aeration, temperature, acidity, moisture, and rate of decomposition in different parts of ordinary flat mushroom compost heaps and that these conditions are distributed in a regular manner that is fairly consistent for heaps of similar size and shape. Perhaps it would be well to point out here the changes in these con-

ditions to be expected from changes in the size and shape of the heap and some of the implications of these phenomena in the general problem of improving the composting practice for mushroom culture.

Considering aeration first, it has been shown that there is a progressive reduction in oxygen accompanied by an increase in carbon dioxide in the interstices of the manure as the center of a compost heap is approached from the outside, and that 8 hours after turning, anaerobic conditions prevail in regions deeper than 1 foot from the top of the heap and more than 3 feet within the side of the heap. Likewise, it is apparent that when ventilators are run under the center of the heap along the ground the anaerobic condition, high in carbon dioxide, in the lower central part of the heap, is changed to a fairly well aerated one. Vertically this change extends from the ventilators to the top of the heap, but laterally the aeration does not seem to extend more than 2 feet. It is evident from these observations that increases in the height or the width of unventilated compost heaps tend to increase the proportion of manure subject to anaerobic conditions over that subject to aerobic conditions. On the other hand, anaerobic conditions can be entirely eliminated by the use of closely spaced ground ventilators.

The distribution of temperature in the compost heap seems to be dependent on three factors, namely, aeration, conduction, and convection. The highest temperatures (160° to 180° F.) are usually confined to a region 2 to 4 feet within the sides of the heap and 1 to 3 feet from the top. The outer layers are cooler because of the lack of insulation from the outside and the lower central region is cooler because the lack of oxygen retards microbial thermogenesis. Since thermogenesis is retarded by a lack of oxygen, changes in the height or width of the heap can be expected to affect the average temperature in much the same way as aeration is changed. Increases in the height or width of the heap reduce the average temperature by increasing the size of the cool central region, and complementary ventilators placed at the ground level materially raise the average temperature of the heap.

A region containing compost having an acid reaction and having a comparatively slow rate of decomposition corresponds roughly with an anaerobic region, and the proportion of compost subject to these conditions is increased also with increases in the height and width of the heap.

It is a common observation that currents of air passing into the sides of compost heaps or through ventilators at the ground level have a tendency to dry out the compost at the sides of the heap and surrounding the ventilators. Therefore, reducing the width of a heap increases the tendency for it to dry out during composting, and the insertion of ventilators at the ground level has a similar tendency.

It would seem then that within reasonable limits decreasing the lateral dimensions of a compost heap, or reducing the width of the heap as compared to the length, tends to increase the proportion of aerated alkaline compost in the heap, to raise the average temperature, and to increase the tendency of the compost to dry out between turnings. The insertion of ventilators at the ground level has a similar effect. On the other hand, increasing the height of the compost heap, as is frequently done when manure is stored for several weeks,

tends to increase the proportion of anaerobic acid compost in the heap, to decrease the average temperature, and to some extent to reduce the tendency toward drying out.

The foregoing considerations naturally raise the question, What composting conditions are likely to produce the most favorable medium for the growth and yield of mushrooms? At the present time this question cannot be answered in a categorical fashion in terms of size, shape, ventilation, and methods of turning the compost heap. Most commercial growers, when using manure of average texture, make up their heaps about 4 feet high, 20 feet wide, and 40 to 60 feet long. In these heaps about one half of the manure composts under anaerobic conditions; and if it were not for the thorough mixing obtained during the turning process, the lower central part of the heap would take 2 or 3 times as long to decompose as the outer portion. Preliminary experiments and the beneficial effect of the final fermentation in the beds suggest that an aerated condition in the compost heap is preferable to an anaerobic condition provided it can be attained without excessive heating or drying out. Theoretically aeration can be increased by making the heaps narrower or lower, by inserting ventilators at the ground level, or, perhaps preferably, by both increasing the height of the heap and inserting ground-level ventilators. Such changes seem worthy of experimental trial, but it should be recalled that they also may tend toward excessive drying out and overheating and that the beneficial effects of aeration are not well established. The problem can probably best be attacked by a series of semi-empirical yield experiments combined with a study of the microbial and insect population encouraged under different conditions. The large number of factors to be considered and the heterogeneity of stable manure, composting conditions, and conditions during the growth of the crop will make sure progress slow and expensive.

As a working hypothesis it may be assumed that composting conditions which produce a favorable medium for the development of mushrooms probably do so because they encourage the development of a microbial population that is best able to pave the way for the subsequent growth and fructification of mushrooms. Such a hypothesis must take full cognizance of the effect of the staling products of different groups of organisms on mushroom development as well as the action of these organisms in producing changes in the manure favorable to the nutrition of mushrooms under competitive conditions. Interesting facts pertinent to the latter question have been brought to light by the culture studies of Styer (*10*, *11*) and Bechman (*2*) and the proximate chemical analyses of Hébert (*5*) and of Waksman and Niessen (*12*).

Raising the temperatures approximately 25° F. at the bottom of the heap by ground-level ventilation suggests interesting possibilities from the standpoint of reducing the introduction of pests into the mushroom houses with the composted manure. If all houses could be properly heated, fumigated during the peak of the heat, and protected thereafter, there would be much less trouble from insect and fungus pests; but at present this ideal is usually not attained, and it is important that the compost be taken into the house as nearly pest-free as possible. In the unventilated heaps a considerable portion of the bottom layer is below 100°, and a still greater proportion below

110°, temperatures that most mushroom pests can survive for some time. It is true that the high carbon dioxide concentration and the lack of oxygen might cause insect and fungus pests to cease activity, but they probably can survive for a long time under those conditions. Temperatures necessary to kill mushroom insects of various species in their various stages have not yet been determined with accuracy but are certainly below 130°. Chapman (*3*) gives 125.6° as the highest authentic record of temperature endured by any insect. In the case of fungus pests the benefits are more uncertain, because some fungus pests are known to withstand temperatures higher than 130°.

SUMMARY

Gas samples taken from all parts of mushroom compost heaps indicate an increase of carbon dioxide and decrease of oxygen toward the lower central part of the heap. In flat heaps 3 feet deep anaerobic conditions are usually found deeper than 1 foot and more than 3 feet from the sides of the heap. Compost in this portion of the heap tends to be acid, while that in well-aerated portions is alkaline or neutral. The highest temperatures (160° to 180° F.) are usually confined to a region 2 to 4 feet from the sides of the heap and 1 to 3 feet from the top. The outer layers are cooler because of the lack of insulation from the outside and the lower central region is cooler because the lack of oxygen retards the microbial activity. At ground level temperatures (100° to 120°) are usually lower than in the higher strata, presumably also because of lack of oxygen. A more uniform distribution of oxygen and wider distribution of the high-temperature region is induced by placing ventilating tiles at ground level. In all probability, conditions such as these influence the suitability of the finished compost for mushroom culture by establishing the trend of the microbial and insect population of the compost heap.

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IRREGULARITIES IN THE INHERITANCE OF THE HAIRY-NECK CHARACTER TRANSPOSED FROM SECALE TO TRITICUM[1]

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INTRODUCTION

A preliminary paper[2] recorded the transfer of the "hairy neck" of rye (*Secale*) to wheat (*Triticum*). This transfer of a definite character is of interest inasmuch as within *Secale* there are certain economic characters, particularly winter hardiness, that are desired in the common wheats. If it can be shown that one character may be successfully transferred from one genus to the other there are good reasons to believe that other characters also may be transferred. This paper deals primarily with the genetic stability and with the behavior of these hairy-neck wheat forms in crosses with different varieties of wheat.

REVIEW OF LITERATURE

Leighty and Taylor[2] and Florell[3] report the isolation of hairy-neck lines from wheat-rye hybrids. Bleier[4] gives a comprehensive review of the work of investigators who have studied phases of the wheat-rye problem. Florell[3] has reviewed studies on the cytology of wheat-rye hybrids.

MATERIALS AND METHODS

Hairy-neck is characterized by the presence of pubescence or hairiness on the peduncle, or that portion of the culm just below the first node of the rachis (fig. 1). In rye plants and in hairy-neck wheatlike segregates from wheat-rye crosses hairiness varies from a few hairs around the apical node of the culm to a dense pubescence extending 3 or more inches below the head.

As reported by Leighty and Taylor,[5] typical wheatlike hairy-neck segregates were selected in 1923 at the Arlington Experiment Farm, near Washington, D.C., from descendants of natural wheat-rye hybrids found in 1918. Ten of these selections were grown originally, but later work was concentrated on three, designated as C, K, and H, which it is believed represent the characteristic behavior of this group of selections.

The three selections are similar to *Triticum vulgare*[6] in their principal spike characters, with the exception of the neck (or peduncle), which is hairy, as shown in figure 2. The plants are not so tall and

[1] Received for publication Nov. 18, 1933; issued June 1934.

[2] LEIGHTY, C. E., and TAYLOR, J. W. "HAIRY NECK" WHEAT SEGREGATES FROM WHEAT-RYE HYBRIDS. Jour. Agr. Research 28: 567–576, illus. 1924.

[3] FLORELL, V. H. A CYTOLOGICAL STUDY OF WHEAT-RYE HYBRIDS AND BACK CROSSES. Jour. Agr. Research 42: 341–362, illus. 1931.

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[5] LEIGHTY, C. E., and TAYLOR, J. W. See footnote 2.

[6] According to the rules of botanical nomenclature the name of this species is *Triticum aestivum*, but as *T. vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writer gives preference to that form.

FIGURE 1.—Necks (upper portions of peduncle) of rye, wheat, and two hairy-neck wheatlike selections from wheat-rye hybrids. *A*, Abruzzes rye; *B*, Selection C; *C*, Selection H; *D*, Fulcaster wheat. × 3. *A*, *B*, and *C* are hairy.

are less vigorous than those of wheat, as is usually the case with hairy-neck segregates of wheat-rye crosses. They are more subject to natural crossing than are commercial varieties of wheat, and selfing has been necessary to maintain them. They may be described as follows:

FIGURE 2.—Heads of wheat and three hairy-neck wheatlike selections from wheat-rye hybrids. *A*, Selection K; *B*, Selection H; *C*, Fulcaster wheat; *D*, Selection C. Natural size.

SELECTION C.—Awnless, white glumes, red kernel, with hairs extending one half inch downward from the apical node.

SELECTION K.—Awned, white glumes, red kernel, with hairs extending one half inch downward from the apical node.

SELECTION H.—Awned, white glumes, red kernel, with hairs extending 4 inches or more downward from the apical node. Spike more lax than that of Selection K.

Observations regarding the stability of each of the three selections with respect to the hairy-neck character were made. Each of these selections was crossed with several varieties of soft red winter wheat grown at the Arlington Farm and the progeny were studied in such a way as to determine the nature of the segregation of the hairy-neck character in relation to other characters of the parents. The F_2 populations were grown in spaced nursery rows and the F_3 populations in 5-foot head rows. All hairy-neck plants of each F_2 population which produced sufficient seed for a test were continued in the F_3 generation. Notes on neck character and height were taken in the field. Data on sterility were obtained from the two lower florets of each spikelet of the primary head.

EXPERIMENTAL RESULTS

CONSTANCY OF THE HAIRY-NECK SELECTIONS

The constancy of the hairy-neck character was determined by selfing hairy-neck plants of each of the selections and recording the number of aberrant types appearing in the following generation. The data for Selection C, which is regarded as typical of the hairy-neck segregates, and which has been selfed since 1925, are given in table 1. It will be seen that Selection C does not stand the test of genetic purity expected of a true species. Of 3,818 plants grown during the 8 years, 262, or nearly 7 percent, were different from those of Selection C with respect to the hairy neck.

TABLE 1.—*Constancy of the hairy-neck character in progeny of Selection C selfed for 8 generations*

Year	Total plants	Plants similar to Selection C	Plants differing from Selection C	
			Variant hairy	Variant smooth
	Number	*Percent*	*Percent*	*Percent*
1924	39	92.3	7.7	0.0
1925	675	93.8	5.9	.3
1926	608	94.7	4.8	.5
1927	388	99.0	1.0	.0
1928	435	93.5	5.1	1.4
1929	227	97.8	1.8	.4
1930	423	91.0	5.9	3.1
1931	1,023	89.2	9.6	1.2
Total or average	3,818	93.1	5.9	1.0

Two aberrant types appeared, one, designated "variant smooth", indistinguishable from wheat, and the other, designated "variant hairy", almost intermediate with respect to hairy neck between Selection C and wheat. There were approximately 1 percent of the former and 6 percent of the latter. Variant smooth is easily distinguished from Selection C because it is smooth-necked and taller. Variant hairy is from 4 to 6 inches taller than Selection C when grown under favorable conditions, but the difference in height may not be apparent under unfavorable conditions. Partly for this reason it is not so readily recognized. However, there is good reason to believe that none of the conclusions arrived at herein is invalidated by errors of classification.

During the 8 years Selection C never behaved as a pure line. Variant hairy necks were found every year and variant smooth necks in 6 of the 8 years. The greatest irregularity occurred in 1931, when 110 plants of a population of 1,023 plants, or 10.8 percent, were variants. The least variation occurred in 1927, when only 1 percent were variants.

Additional data regarding the constancy of Selection C and the breeding behavior of the variants selected from it were obtained by growing in 1930 a selfed plant of Selection C and selfing the progeny and growing them in 1931. The pertinent data are presented in table 2.

The progeny of the single selfed plant in 1930 were classified as 50 similar to Selection C, 1 variant hairy, and 2 variant smooth. Only 49 of the 50 plants of Selection C indicated in table 2 were grown in 1931, 1 failing to produce sufficient seed. Each of the 49 plant rows supported the 1930 classifications, breeding typical Selection C with 7.3 percent variants. The 2 variant smooth-neck plants bred smooth, and the variant hairy-neck plant segregated in the ratio of 3 smooth to 1 hairy.

TABLE 2.—*Breeding behavior of the hairy-neck character in the progeny of a plant of Selection C during 2 generations of selfing, 1930 and 1931*

Progeny from selfed plant, 1930		Progeny from second generation of selfing, 1931			
Type	Total plants	Total plants	Type of plant		
			Selection C	Variant hairy	Variant smooth
	Number	*Number*	*Percent*	*Percent*	*Percent*
Selection C	50	384	92.7	7.0	0.3
Variant hairy	1	8	.0	25.0	75.0
Variant smooth	2	26	.0	.0	100.0

The percentage of smooth-neck plants in this particular line of Selection C in 1930 and 1931 was somewhat less (0.7 percent), and the proportion of variant hairy plants slightly more (6.4 percent) than the average shown in table 1.

During this study of the inconstancy of Selection C 30 variant hairy-neck plants were grown in head or plant rows. These produced 1,388 plants of which 321, or 23.1 percent, were hairy-necked and 76.9 percent smooth-necked, thus approximating the results, presented later, of crosses between Selection C and wheat.

The average proportion of smooth-neck plants appearing in Selection C, that is, about 1 percent, may be explained by assuming a simultaneous loss of the hairy-neck factor in 10 percent of the pollen cells and egg cells. The expected proportion of smooth-neck, variant hairy-neck (heterozygous), and Selection C types is then given by the expansion of the binomial $(1+9)^2$. The fact that the smooth-neck plants bred true and the hairy-neck plants bred like F_1 hybrids is in accord with this hypothesis. However, the average proportion of variant hairy-neck plants, approximately 6 percent, is only about one third of the number to be expected on this basis. It seems necessary to assume also differential functioning or vigor of the two types of gametes

or zygotes, or it is possible that the loss of the hairy-neck factor may occur in a somatic division in the development of the primordium for the flowers of a spike.

INHERITANCE IN CROSSES OF HAIRY-NECK SELECTIONS × WHEAT

Hybridizing the hairy-neck selections with common wheat may be expected to give further information as to the genetic irregularity of the hairy-neck character and its relation to the inheritance of certain common wheat characters. In 1923 and later, selections which from phenotypic indications were pure for the hairy-neck character of the C, K, and H selections, were crossed with common wheat varieties. The varieties chosen differed in such head characters as awnlessness and awnedness, red and white glume color, and smoothness and pubescence of glumes. The segregation of the common allelomorphs permitted observation as to the effect of an intergeneric character on their behavior.

The F_1 hybrids conformed in the more common spike characters to what would be expected in crosses of wheat varieties; that is, there was expressed the incomplete dominance of awnlessness, red glume color, and pubescent chaff. The hairy-neck character was dominant, but the hairs did not extend downward so far as in the parental selection, and the density of the hairiness was decidedly reduced. The F_1 heads appeared fully fertile and were selfed.

The hairy-neck selections were crossed with one or more of the varieties of *Triticum vulgare*, namely, Brown Fife,[7] Purplestraw, Fulcaster, Nittany, Poole, and Fultz. All except Fulcaster and Nittany are awnless, and all except Brown Fife and Poole have glabrous white glumes. Brown Fife has pubescent red glumes, and Poole has glabrous red glumes.

Glume color developed poorly, and segregates from this character were not classified, although it was evident that hairy neck was present in both the red- and white-glume segregates of the F_2 generation. The number of F_1 plants secured in each case, the number of F_2 plants that were grown, and the segregation of the latter with respect to presence of awns, pubescence of glumes, and hairy necks are shown in table 3.

The segregation with respect to awns and pubescence is what would be expected when varieties of *Triticum vulgare* possessing these characters are crossed. In the six crosses involving awn segregation, the fully awned recessive constitutes 24.4 percent of the total number of plants that were grown. In the single cross (Selection C × Brown Fife) involving pubescent and glabrous glumes, 24.1 percent of the plants had glabrous glumes. On the other hand, the segregation with respect to the hairy-neck character was quite irregular. In the five crosses involving Selection C the percentage of hairy-neck plants ranged from 17.7 to 29.2 and averaged 25. In the four crosses involving Selection K, the percentage of hairy-neck plants ranged from 30.6 to 48.2, with an average of 36.2. There were two crosses involving Selection H. In these the percentages of hairy-neck plants were 61 and 63.4, respectively, averaging 62.9.

[7] The name "Brown Fife" was given in 1922 to a strain of wheat formerly grown as Jones Winter Fife. In habit of growth and morphological characters it is somewhat similar to Grandprize.

TABLE 3.—*Segregation in the F_2 generation from crosses of 3 hairy-neck selections with varieties of common wheat at the Arlington Experiment Farm, Rosslyn, Va.*

Cross	F_1 plants	F_2 plants	F_2 plants of indicated class					
			Awnless				Awned (glabrous)	
			Pubescent		Glabrous			
			Hairy	Smooth	Hairy	Smooth	Hairy	Smooth
	Number	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Selection C × Brown Fife	1	220	12.3	63.6	5.5	18.6	0.0	0.0
Selection C × Purplestraw	2	1,365	.0	.0	26.4	73.6	.0	.0
Selection C × Fulcaster	1	168	.0	.0	22.6	57.7	6.6	13.1
Nittany × Selection C	12	343	.0	.0	15.2	58.6	5.8	20.4
Selection C × Poole	1	171	.0	.0	26.9	73.1	.0	.0
Selection K × Purplestraw	4	486	.0	.0	37.7	39.5	10.5	12.3
Selection K × Fultz	13	281	.0	.0	23.8	50.2	8.2	17.8
Fulcaster × Selection K	1	191	.0	.0	.0	.0	38.7	61.3
Poole × Selection K	8	950	.0	.0	21.9	52.1	8.7	17.3
Selection H × Fultz	1	100	.0	.0	51.0	27.0	10.0	12.0
Selection H × Fulcaster	9	383	.0	.0	.0	.0	63.4	36.6

In none of the crosses involving Selections K and H do the ratios conform to simple Mendelian inheritance. The average results for Selection C agree exactly with expectations for a monohybrid, except that hairy neck behaves as the recessive, whereas in the F_1 this character was dominant. The breeding behavior of the F_1 of Selection C × wheat is similar to that of the hairy-neck variants.

There is no indication of linkage of the hairy-neck character with either of the other characters studied except in the Selection H × Fultz cross, in which the proportion of hairy necks in a small population is approximately twice as great for the awnless segregates as for the awned.

A number of the crosses were continued into the F_3 generation. Some of these were space-planted, but the greater number were grown in 5-foot head rows. In some cases all the plants from the F_2 rows were grown, whereas in others only the hairy-neck plants were grown. The progeny of 388 smooth-neck F_2 plants were grown and all bred smooth neck. The data for the hairy-neck plants are presented in table 4.

Of the 125 F_3 lines grown from hairy-neck F_2 plants of the two crosses of Selection C, only 3, or 2.4 percent, were homozygous. If the hairy-neck character were a simple recessive, 33.3 percent should be homozygous.

Of the 83 hairy-neck F_2 plants of the cross Selection K × Fultz grown in the F_3, approximately 11 percent were homozygous hairy neck. However, of the 76 F_3 lines of the cross Selection K × Purplestraw 25 percent were homozygous. In the F_2 of Selection K × Purplestraw approximately 50 percent of the plants were hairy neck as compared to 32 percent in the cross Selection K × Fultz. In the former cross the F_2 homozygous hairy-neck plants appeared more than twice as often as in the latter cross.

TABLE 4.—*Breeding behavior of hairy-neck F_2 plants from crosses of hairy-neck selections × wheat*

Cross	F_3 lines		
	Number	Heterozygous hairy neck	Homozygous hairy neck
		Percent	*Percent*
Selection C × Purplestraw	71	98.6	1.4
Nittany × Selection C	54	96.3	3.7
Selection K × Fultz	83	89.2	10.8
Selection K × Purplestraw	76	75.0	25.0
Selection H × Fultz	54	81.5	18.5

The F_1 of Selection K × Fultz was grown in 1925 and the F_1 of Selection K × Purplestraw in 1928. The difference in percentage of homozygosity of the two crosses is believed to be due to differences in the two seasons, inasmuch as 30 of the segregating F_3 lines of Selection K × Purplestraw, involving 545 plants, were space-planted and 25.5 percent of the plants were hairy as compared to approximately 48.1 percent in the F_2 generation. The higher percentage of hairy-neck plants in the F_2 generation of this cross as compared to that of the other three crosses of Selection K × wheat, and the comparatively high percentage of F_3 homozygous hairy-neck lines, indicate conditions unusually favorable for either the formation or the functioning or both of the hairy-neck gametes of the F_1 plants.

Of the 54 F_3 lines of Selection H × Fultz, 18.5 percent were homozygous hairy-neck. Selection H crosses produced approximately 61 percent of hairy-neck plants in the F_2; that is, the hairy-neck character behaved as dominant. However, the F_3 test clearly shows a lethal factor operating to eliminate the homozygous hairy-neck type.

EFFECT OF HAIRY NECK ON PLANT CHARACTERS

The supposition of a lethal effect of the hairy-neck factor suggested the desirability of a study of sterility, seed germination, height of plant, and tillering of the crosses, especially with respect to the smooth-neck and hairy-neck segregates.

STERILITY

The percentages of sterile florets of the hairy-neck selections and of the F_1 hybrids between these and certain varieties of wheat are shown in table 5.

TABLE 5.—*Floret sterility of hairy-neck selections and of F_1 hybrids of hairy-neck selections × wheat*

Selection or F_1 hybrid	Florets	Seeds	Sterile florets
	Number	*Number*	*Percent*
Selection C	1,190	789	33.7
Nittany × Selection C	369	321	13.0
Selection C × Purplestraw	1,356	1,249	7.9
Selection K	282	159	43.6
Selection K × Fultz	536	487	9.1
Selection H	204	160	21.6
Selection H × Fultz	106	102	3.8

In Selection C, 33.7 percent of the florets were sterile, and in the F_1 hybrid of Selection C × wheat and its reciprocal, approximately 10 percent of the florets were sterile. This is about the average sterility for wheat. The average sterility of Selection K was 43.6 percent and of Selection H, 21.6 percent. The F_1 hybrids with wheat in each case were as fertile as would be expected for wheat, the sterility of Selection H × wheat being only 3.8 percent. Selection H has the highest fertility of the three selections and the same relation exists between the F_1 hybrids with wheat. It is pertinent in this connection to note that in the F_2 generation 61 percent of the plants of this cross had hairy necks as compared with 25 and 36 percent in Selections C and K, respectively (table 3). In Selection H, hairiness extends 4 inches down the peduncle as compared to approximately one half inch in the other two selections; that is, the degree of hairiness in these selections was not positively correlated with reduction of fertility as might be expected.

Seed Germination

Seed of the F_1 plants of wheat × Selection C was planted and allowed to mature. Approximately 85 percent of the planted seeds matured plants. A similar study was made of Selection K × wheat from F_2

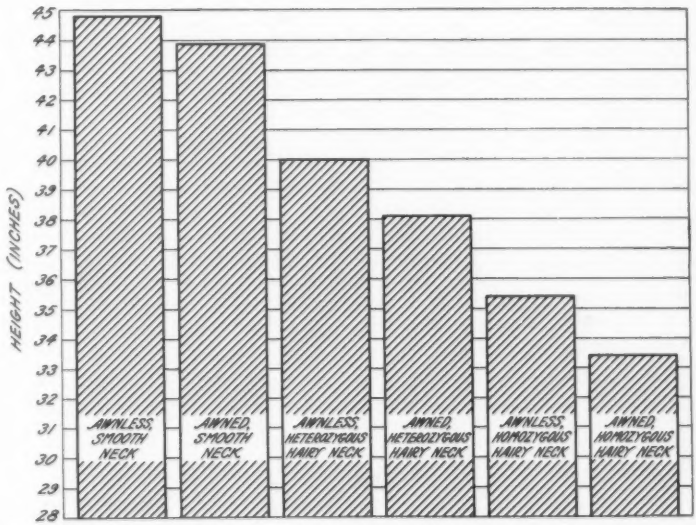

Figure 3.—Height of plants of different awn and neck types in the F_2 generation of the cross Selection K × wheat.

seed, as no F_1 seed was available. Conditions for germination were poor, and only 49 percent of the seed of segregating hairy-neck lines and a like percentage of homozygous hairy-neck lines matured plants. A similar planting of smooth-neck seed from the same cross matured only 48 percent of plants. In neither case is there any evidence of differential zygotic lethals.

Height of Plant

The height of a large number of plants in the F_2 and F_3 generations was measured, and the data are presented in table 6. In each of the 17 possible comparisons of the hairy-neck with the smooth-neck classes the hairy-neck plants were significantly shorter, in most cases materially so. The average height of the smooth-neck plants was 46.9 inches as compared to 41.4 inches for the hairy-neck plants. Furthermore, the homozygous hairy-neck F_3 lines were approximately from 2 to 5 inches shorter than those segregating for hairy neck. The comparative height differences in the classes obtained from the cross of Selection K × Fultz are shown graphically in figure 3. No significant differences were found between the height of the plants as a result of the presence or absence of awns.

The commonly cultivated rye varieties have hairy necks. A few strains of smooth-neck rye have been bred, the height of which is no greater than that of their hairy-neck relatives. It is probable, therefore, that the hairy-neck character in the presence of the rye-chromosome set does not adversely affect the height of the plant. The average height of the rye varieties grown at the Arlington Experiment Farm varies from 62 to 65 inches as compared with 46 and 54 inches in the wheat varieties.

Table 6.—*Average height of hairy-neck and smooth-neck plants in heterozygous and homozygous hairy-neck lines from hybrids of hairy-neck selections × wheat*

Class and hybrid	Generation	Plants	Average height of plants of indicated class			
			Awnless smooth	Awnless hairy	Awned smooth	Awned hairy
HETEROZYGOUS		*Number*	*Inches*	*Inches*	*Inches*	*Inches*
Selection K × Fultz	F_2	281	47.5±0.42	40.7±0.39	47.7±0.40	38.7±0.85
Do	F_3	1,466	44.8± .36	40.0± .38	43.9± .47	38.1± .57
Selection C × Purplestraw	F_2	1,172	44.1± .39	39.6± .45		
Selection H × Fultz	F_2	90	47.5± .55	42.8± .66	47.6± .69	40.9±1.83
Do	F_3	443	49.1± .14	45.2± .16	49.8± .11	46.9± .16
HOMOZYGOUS LINES						
Awnless hairy (Selection C × Purplestraw)	F_3			36.1± .44		
Awned hairy (Selection K × Fultz)	F_3					33.4± .80
Awnless hairy (Selection K × Fultz)	F_3			35.4± .97		
Awned hairy (Selection H × Fultz)	F_3					44.8± .80
Awnless hairy (Selection H × Fultz)	F_3			43.2± .72		

Tillering

Data on tillering were obtained for individual plants in the F_2 generation of the two crosses Selection K × Fultz and Selection H × Fultz. The former was grown on more productive land than the latter. In all cases the smooth-neck plants tillered more than did the hairy-neck plants (table 7). The differences between awned and awnless plants were not statistically significant.

TABLE 7.—*Average number of tillers per plant in F_2 classes of hairy-neck selections × wheat crosses*

Cross	Average tillers per plant in indicated F_2 class					
	Awnless			Awned		
	Hairy	Smooth	Difference	Hairy	Smooth	Difference
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Selection K × Fultz	6.7±0.39	8.5±0.46	1.8±0.60	6.6±0.58	9.5±0.46	2.9±0.74
Selection H × Fultz	3.7± .12	5.3± .30	1.6± .32	3.4± .37	4.5± .49	1.1± .61

BACK-CROSSING F_1 HYBRIDS WITH WHEAT

Since no evidence of zygotic elimination was obtained it seemed desirable to resort to back-crossing to test for comparative functioning of male and female gametes carrying the smooth-neck and hairy-neck factors. This was done by reciprocally crossing the F_1 hybrids with wheat, only the F_1 hybrids of Selection C and Selection K being used. The resulting progeny were then classified with respect to the hairy-neck character. Errors due to self-pollinated plants were eliminated by the selection of a wheat variety in which selfing could be detected. The data are presented in table 8.

The female gametes of Selection K × Fultz, fertilized by wheat pollen, produced plants of which 16.9 percent had hairy necks, whereas the male gametes of the same hybrid, fertilizing wheat egg cells, produced but 9.0 percent of hairy-neck plants. Similarly, the female gametes of the F_1 of Selection C × wheat (Purplestraw and Nittany), fertilized by wheat pollen, produced 13.2 percent of hairy-neck plants, whereas the male gametes of the same hybrid, fertilizing wheat egg cells, produced 8.9 percent of hairy-neck plants. In all back crosses except one a larger percentage of hairy-neck plants was produced when the F_1 hybrid was used as the female parent. However, neither the functional male nor female gametes carried the hairy-neck character in the expected frequency, since in back-crossing experiments such as these the hairy-neck and smooth-neck gametes should occur in equal numbers.

TABLE 8.—*Hairy-neck and smooth-neck plants resulting from reciprocal back-crossing of the F_1 hybrid of hairy-neck selections × wheat with wheat*

F_1 hybrid	Year	F_1 hybrid as the female produced—			F_1 hybrid as the male produced—		
		Smooth-neck plants	Hairy-neck plants		Smooth-neck plants	Hairy-neck plants	
		Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Selection K × Fultz	1925	23	6	20.7	17	3	15.0
Do	1926	46	8	14.8	54	4	6.9
Total or percent		69	14	16.9	71	7	9.0
Selection C × Purplestraw	1926	34	6	15.0	46	3	6.1
Nittany × Selection C	1929	18	5	21.7	6	0	0
Selection C × Purplestraw	1930	93	11	10.6	92	11	10.7
Total or percent		145	22	13.2	144	14	8.9

Theoretically, there should be agreement among the F_2 segregation, the F_3 family behavior (that is, whether homozygous or heterozygous for the neck character), and the results from the back crosses. The latter indicated that approximately 16.9 percent of the functional eggs and 9.0 percent of the male cells of Selection K × Fultz (table 8) carry the hairy-neck factor. Assuming the same gametic frequency (1+5) (1+10) and the same functioning in the selfed F_1 hairy neck × wheat hybrids, the F_2 population should contain 24.2 percent of hairy-neck plants, and approximately 6.3 percent of these should be homozygous in F_3. Actually, 32 percent of the F_2 plants were hairy (table 3), and 10.8 percent of the F_3 lines were homozygous (table 4).

In Selection C × wheat slightly more than 13 percent of the egg cells and about 9 percent of the male cells carried the hairy-neck character. On this basis the F_2 population from the selfed F_1 hybrids should be approximately 21.0 percent hairy neck, and 5.7 percent of these should be homozygous. The percentage of the hairy-neck plants actually observed in the F_2 generation of the same crosses (Purplestraw and Nittany) was 25.3 (table 3), and 2.4 percent of these bred true (table 4).

The agreement between the data of the different experiments is perhaps as close as should be expected, considering the small populations obtained from the back crosses and the apparent irregularity in genetic expression due to environment.

DIFFERENTIAL FUNCTIONING OF POLLEN CELLS

The low percentage of functional gametes carrying the hairy-neck factor, as shown in the reciprocal back crosses of the F_1 hybrids of hairy-neck selections × wheat with wheat, would indicate elimination of the hairy-neck character at meiosis or at fertilization. The high percentage of fertility in the F_1 hybrids of hairy-neck selections × wheat favors the assumption that functional female gametes carrying the hairy-neck factor are not formed in the expected frequency. The apparent differential functioning of the male gamete of the F_1 hybrids carrying the hairy-neck and smooth-neck characters (table 8) may, however, be due to a growth differential between the two types rather than to nonformation of pollen cells carrying the hairy-neck factor. Experiments were therefore made with mixtures of pollen of Selection C and pollen of three varieties of common wheat, namely, Nittany, Dixie, and Red Rock. Heads of the wheat or of Selection C were emasculated and at the proper time were pollinated with a pollen mixture or first with pollen of Selection C and then with pollen of the wheat variety; in the latter case the interval between the two pollinations averaged about 2 minutes. The pollen mixture was composed of the contents of the same number of anthers of Selection C and of the wheat variety. The anthers of Selection C are larger than those of the wheat varieties used.

When wheat was the female, the progenies were grown and classified as smooth neck or hairy neck, the results showing which pollen grain functioned. When Selection C was the female, glume color or awn contrast of the following progenies showed when the wheat pollen grain functioned, except in Dixie, when the plants were carried to the F_2 generation to identify them. Results of the pollinations are shown in table 9.

TABLE 9.—*Comparative functioning of pollen of hairy-neck selection C and wheat varieties in pollen-mixture and double-pollination experiments*

Female parent	Year	Pollen source	Plants of indicated type resulting from pollination			Flowers fertilized by pollen carrying hairy neck
			Wheat	Selection C	Hybrid	
			Number	*Number*	*Number*	*Percent*
Nittany	1928	Mixture Nittany and Selection C	10		1	
Dixie	1928	Mixture Dixie and Selection C	11		0	
Red Rock	1929	Mixture Red Rock and Selection C	17		2	
Nittany	1928	Selection C and Nittany	11		3	
Dixie	1928	Selection C and Dixie	12		1	
Red Rock	1929	Selection C and Red Rock	42		5	
Total			103		12	10.4
Selection C	1928	Mixture Dixie and Selection C		2	22	
Do	1928	Selection C and Nittany		2	14	
Do	1929	Selection C and Dixie		0	7	
Do	1929	Selection C and Red Rock		1	8	
Total				5	51	8.9

One hundred and fifteen plants resulted from pollinating the common wheat varieties with pollen from the two sources. Only 12, or 10.4 percent, were hairy-neck hybrids, the remainder being selfs. When the wheats were pollinated with the mixture the percentage of hybrids was 7.3, and when pollinated first with pollen of Selection C and then selfed the percentage of hairy-neck hybrids increased to 12.2, possibly indicating an effect due to rate of pollen germination or of pollen-tube growth.

Fifty-six plants were secured in the experiments in which Selection C was the female. Fifty-one, or 91 percent, proved to be hybrids and only 5, or 9 percent, were selfs. Approximately the same number of flowers of wheat and of Selection C were pollinated in these experiments, and the fewer seeds and plants obtained indicates again the sterility of Selection C as compared with that of wheat. These results suggest that the pollen cells of Selection C which carry the hairy-neck factor are less viable or that the pollen tube grows more slowly than that of normal wheat. Poor functioning of pollen cells carrying the hairy-neck factor appears at least as probable as nonformation at meiosis in the F_1 hybrid. This is further supported by the agreement between the results from back-crossing the F_1 of Selection C $\times$ wheat with wheat (table 8) and the results from pollinating wheat and Selection C with the pollen mixture. Wheat fertilized with pollen from the F_1 hybrid (Selection C $\times$ wheat) produced 8.9 percent of hairy-neck plants, whereas wheat fertilized with a mixture of pollen from Selection C and wheat produced 10.4 percent of hairy-neck plants; and Selection C fertilized by a mixture of pollen from Selection C and wheat produced 8.9 percent of selfed hairy-neck plants (table 9).

DISCUSSION

The genetic behavior of the hairy-neck wheatlike selections isolated from wheat-rye hybrids shows that the addition of the rye character results in an unbalanced type. Hairy-neck is a tangible rye character transposed to types that are apparently otherwise *Triticum vulgare*. A preliminary cytological examination of one of the hairy-neck plants

made by Florell [8] showed 44 chromosomes in the root tips as compared to 42 for *T. vulgare*. Inasmuch as the hairy-neck plants are not constant, their chromosomal constitution seems to be better represented by the quantitative expression $2n+2$ rather than $2n$, indicating in this case no homologue in the wheat complement for the rye chromosome. Blakeslee [9] uses the formula $2n+2$ for one of his Globe mutants in *Datura* where the unbalance was of a simple tetrasomic type.

To explain the genetic behavior of the hairy-neck character it may be assumed that the $2n+2$ hairy-neck plants normally produce $n+1$ gametes but that occasionally in male and female gametogenesis the rye chromosome is lost, giving a gamete of n constitution. The fertilization of $n+1 \times n$ gametes results in a zygote similar in later behavior to the cross hairy-neck selection × wheat, whereas the mating of $n \times n$ gives a zygote which produces a plant indistinguishable from *Triticum vulgare*.

The chromosome number of the F_1 hybrid hairy-neck selection × *Triticum vulgare*, and also of the variant hairy type, would be $2n+1$ and the plants would be of the hairy-neck type as the character is dominant over the smooth neck. In gametogenesis and fertilization, irregularities in the functioning of $n+1$ and n gametes apparently occur, as the F_2 segregation often shows the hairy character as recessive, and results from the back crosses indicate that from 13 to 17 percent of the functional egg cells and approximately 9 percent of the functional pollen grains carry the hairy character. Furthermore, F_3 lines homozygous for hairy neck do not appear in the expected frequency even for a recessive character. Reduced height and tillering and varying degrees of sterility in the plants with hairy neck as compared to those with smooth neck, in addition to the genetic irregularities, support the belief that there is incompatibility between the wheat and rye complexes and that the reaction is unfavorable both to the normal productiveness of the plant and to its constancy in breeding. Whether the addition or substitution of other rye chromosomes in the wheat complement would react similarly is questionable. Wheat-rye hybrids carrying all the chromosomes in both wheat and rye, $2n=56$, have been produced,[10] but the economic value of such plants has not seemed particularly promising in the United States. Wheat breeders in general are interested in obtaining a definitely *T. vulgare* type with certain desired rye characters rather than a type intermediate between the two genera.

SUMMARY AND CONCLUSIONS

Complete genetic balance has not been obtained in three hairy-neck selections of wheat × rye crosses designated as Selection C, Selection K, and Selection H. In spite of continuous selfing, approximately 1 percent of the plants of Selection C had smooth necks and bred true and about 6 percent had hairy necks and bred in the same manner as the F_1 hybrids.

The observed proportion of smooth-neck plants may be explained by assuming a simultaneous loss of the hairy-neck factor in 10 per-

[8] Letter addressed to J. W. Taylor by V. H. Florell, Feb. 28, 1931.

[9] BLAKESLEE, A. F. VARIATIONS IN DATURA DUE TO CHROMOSOME NUMBER. Amer. Nat. 56: 16-31, illus. 1922.

[10] LEVITSKY, G. A., and BENETZKAIA, G. K. CYTOLOGICAL INVESTIGATIONS OF CONSTANT INTERMEDIATE RYE-WHEAT HYBRIDS. (PRELIMINARY COMMUNICATION.) U.S.S.R. Cong. Genet., Plant and Animal Breeding, Proc. 2: 345-352, illus. 1930. [In Russian. English Summary, pp. 350-352.]

cent of the pollen cells and egg cells, but the observed proportion of hybrid hairy-neck plants has been only about one third of the number to be expected on the basis of this explanation. It seems necessary to assume also differential functioning or vigor of the two types of gametes or of the zygotes, or possibly loss of the hairy-neck character in somatic mitosis.

In crosses between the three selections and several varieties of wheat the hairy-neck character appeared to be dominant, but in later generations it behaved as a recessive or in an irregular manner.

There appeared to be no linkage of the hairy-neck character with glume color, with condition of glumes in regard to pubescence, or with condition of heads in regard to awns.

In studies of sterility it was found that Selection C, Selection H, and Selection K were materially less fertile than wheat, but that the F_1 hybrids were approximately as fertile as wheat. There was no observable inverse relation between degree of hairiness and sterility, as might be expected; Selection H, which had more hair on the necks than the others, was the most fertile.

In a comparison of the germination of segregating hairy-neck lines, homozygous hairy-neck lines, and homozygous smooth-neck lines, no differences were observed.

In study of the height of plants and of vigor as measured by tillering, it was found that in crosses between the three selections and wheat the smooth-neck segregates were invariably taller than the hairy-neck segregates from the same cross. It was also found that heterozygous hairy-neck segregates were taller than homozygous hairy-neck segregates. In all cases smooth-neck plants from these crosses tillered more than comparable hairy-neck plants.

The F_1 hybrids were reciprocally back-crossed with wheat. In all crosses but one, a larger percentage of hairy-neck plants was produced when the F_1 hybrid was used as a female parent. In the one exception there was practically no difference. There was good agreement among the data secured by back-crossing, the F_2 segregation, and the breeding behavior of the F_3 lines.

A study of differential functioning of pollen grains was made by using mixtures of pollen of Selection C and one of three varieties of wheat. The florets were emasculated and either pollinated with a mixture of pollen or pollinated first with pollen from Selection C and about 2 minutes later with pollen from wheat. The results indicated that the pollen cells of Selection C are less viable or that the pollen tube grows more slowly than in wheat. There was apparently no discrimination on the part of the egg toward either type of gamete.

Since the hairy-neck plants are irregular in their breeding behavior, it seems logical to represent their chromosomal constitution by the expression $2n+2$ rather than by $2n$, indicating no homologue in wheat for the rye chromosome carrying the hairy-neck factor. It may then be assumed that the hairy-neck plants produce $n+1$ gametes and that occasionally the rye chromosome is lost, giving a gamete of n consitution. The union of $n+1$ and n gametes results in a zygote similar to that produced by a cross of a hairy-neck selection $\times$ wheat, and the union of n gametes produces a plant which cannot be distinguished from wheat.

THE COMPARATIVE EFFECTIVENESS, IN THE DAIRY RATION, OF SUPPLEMENTS OF PHOSPHORUS IN THE FORM OF ORTHOPHOSPHORIC ACID, MONOSODIUM, DISODIUM, TRISODIUM PHOSPHATES, AND BONE MEAL [1]

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INTRODUCTION

Studies of calcium and phosphorus metabolism carried on for a number of years at the United States Dairy Experiment Station at Beltsville, Md., have shown that a proper adjustment of the mineral content of the ration is of vital importance to health, milk production, and reproduction in the dairy cow.

The work of Shohl (*5*)[2] with rats a few years ago suggested the possibility that the assimilation of calcium and phosphorus might be affected by a change in the acid-base ratio of the ration. Shohl found the greatest retention of calcium and phosphorus in rats on a neutral diet. The neutral diet was obtained by adding orthophosphoric acid to an alkaline ration. The alkaline ration alone produced the symptoms of tetany and the acid ration (made by adding phosphoric and hydrochloric acid to the alkaline ration) produced the symptoms of rickets.

One of the writers (*6*) has shown that for favorable assimilation of calcium and phosphorus by dairy cows the calcium-phosphorus ratio should not be too wide. Dairy rations of alfalfa hay and grain often have a rather large proportion of calcium as compared with phosphorus.

The experiments here reported were undertaken (1) to study the effect of variations in the alkalinity of the ration on the calcium and phosphorus metabolism in cows, and (2) to determine the form in which supplements of phosphorus could best be supplied. In the first experiment the ration was supplemented with soluble phosphates; in the second experiment bone meal was used. An attempt was made to eliminate the effect of any organic food constituents by feeding a uniformly good quality of hay and grain throughout.

FIRST EXPERIMENT: ORTHOPHOSPHORIC ACID, MONOSODIUM, DISODIUM, AND TRISODIUM PHOSPHATES AS SUPPLEMENTS OF PHOSPHORUS

In the first experiment a basal ration somewhat low in phosphorus was used and phosphorus supplements were added in the form of orthophosphoric acid, monosodium, disodium, and trisodium phosphates. These were added in such amounts as to maintain a calcium-

[1] Received for publication Nov. 23, 1933; issued June, 1934.
[2] Reference is made by number (italic) to Literature Cited, p. 630.

phosphorus ratio in the feed below 1.50, preferably about 1.25. Under such conditions it was hoped that some superiority of one form of supplement over another would be apparent.

Calculated, according to Shohl, on the basis of inorganic acids and bases in the ration, 100 grams of this basal ration was equivalent to 63.3 cubic centimeters normal alkali. The amount of phosphoric acid added was in no case sufficient to neutralize the alkalinity. This method of calculating the reaction of the ration, however, disregards carbonates and organic acids. It may be said to give a rough idea of the titratable alkalinity of the ration, but it gives no idea of what the hydrogen-ion concentration would be in the kind of watery extract that is formed when such a ration is introduced into the alimentary tract. The amounts of orthophosphoric acid and of trisodium phosphate used in this experiment may well have been sufficient to produce definite changes in the hydrogen-ion concentration of the alimentary contents in the early stages of digestion.

The provision for additional phosphorus, at least in the form of the more neutral supplements, has proved valuable and the results have led to certain conclusions which will be discussed later.

EXPERIMENTAL PROCEDURE

For this experiment three Holstein cows were used. Cow 265 was a purebred, and cows A–37 and A–40 were grades. Cows A–37 and A–40 were about 4 years old, and cow 265 was 9 years old. All three cows were pregnant and in the fourth month of lactation when the experiment was started, but cows A–37 and 265 aborted early in the experiment after about 2 months of pregnancy. They were bred again and, at the end of the experiment, cow A–37 had completed 5 months of pregnancy; cow A–40, 7 months; and cow 265, 1.5 months.

The experiment began September 20 and ended March 13, a period of 25 weeks. During the first 4 weeks a basal ration was fed, consisting of U.S. No. 1 grade alfalfa hay and a grain mixture (whole yellow corn meal, 40 parts; wheat bran, 30 parts; soybean meal, 20 parts; linseed meal, 10 parts; and sodium chloride, 1 part). The cows were given as much feed as they would "clean up" and an effort was made to maintain about equal consumption of grain and hay.

Because of the hot weather at this time (early fall) it was difficult to induce the animals to consume sufficient feed to meet their energy requirements, particularly in the case of cow 265. This cow was offered a little timothy hay. She seemed to relish it, and since it had been observed in other experiments at this station (*2*) that animals at times indicated a preference for timothy hay after prolonged periods of alfalfa-hay feeding, it was decided to give all the cows a feeding period on mixed timothy and alfalfa hay. Accordingly, for the next 4 weeks, half of the alfalfa hay of the basal ration was replaced by U.S. No. 1 grade timothy hay. During the following 2 weeks the basal ration was again fed.

During the last 15 weeks of the experiment different phosphorus supplements (equivalent to about 25 to 27 grams of phosphorus daily) were added to the basal ration for periods of 3 weeks each, to learn the effect of varying the alkalinity of the ration.

Beginning with the ninth week, the cows were exercised 10 minutes daily until the end of the experiment. At about that time their appetites began to improve and subsequently their rate of food con-

sumption became steadier. This may be attributed to a combination of factors—exercise, cooler weather, and possibly the feeding of phosphorus supplements.

The weights of the cows at the beginning and end of the experiment were, respectively: Cow A–37, 561 and 651 kilograms; cow A–40, 504 and 577 kilograms; cow 265, 634 and 604 kilograms. The loss of weight by cow 265 was due to the fact that she would not eat sufficient feed to meet her energy requirements.

Hay and grain were fed twice a day and the cows were milked three times a day. Detailed analyses of the feeds are omitted. The alfalfa hay contained approximately 1.5 percent calcium, 0.2 percent phosphorus, and 2.4 percent nitrogen. The timothy hay contained about 0.35 percent calcium, 0.12 percent phosphorus, and 0.82 percent nitrogen. The average grain mixture contained about 0.13 percent calcium, 0.7 percent phosphorus, and 3.4 percent nitrogen. The phosphorus content of the grain was increased by the addition of phosphorus supplements, and was then between 0.9 and 1 percent.

Chemically pure materials were used as supplements and were mixed with the grain. Three parts of sirupy orthophosphoric acid were diluted with 2 parts of water and dropped on the grain as fed each day. During the last 3 weeks of the experiment the phosphoric acid was thoroughly kneaded into the grain mixture to insure actual consumption of the acid.

EXPERIMENTAL RESULTS

Table 1 shows the average weekly feed consumption, milk yield, percentage of calcium and phosphorus in the milk, and the calcium and phosphorus balances for each cow during the different feeding periods. The figures for assimilated calcium and phosphorus were calculated as described in a previous publication (*3*).

Both calcium and phosphorus values indicate that the different feeds affected the composition of the milk, probably through changes in the composition of the blood. The phosphorus content of the milk shows a fairly definite tendency to be a little higher during the periods in which the phosphate supplements were fed than at other times. The calcium in the milk is noticeably higher when the cows were on the basal ration than when grain, alfalfa, and timothy were fed, and also shows a tendency to be higher on the orthophosphoric acid supplement than on trisodium phosphate. In the case of cow A–40 this latter tendency is partly masked by the general tendency for the milk calcium to increase during the latter part of lactation, due perhaps to the decreasing milk yield.

The graphs in figure 1 show the fluctuation in the calcium and phosphorus content of the body which occurred during the course of the experiment. The calcium and phosphorus graphs are drawn on different scales, in the ratio of calcium to phosphorus in bone. A variation of 100 grams of calcium corresponds to a variation of 46 grams of phosphorus. If the calcium and phosphorus balances signify a building up or breaking down of bone material only in the body, then the graphs should follow an identical course. The fact that considerable divergence is shown between the calcium and phosphorus graphs indicates some difference in the storage possibilities of these two elements in the body, or in the intestinal tract.

TABLE 1.—*Average weekly feed consumption, milk yield, percentage of calcium and phosphorus in the milk, and the calcium and phosphorus balances for the 3 cows during the different feeding periods*

Ration, length of feeding period, and cow no.	Feed consumed		Milk produced			Calcium					Phosphorus					Calcium-phosphorus ratio in feed
				Composition		In urine and feces	In milk	In feed	Balance	Assimilation	In urine and feces	In milk	In feed	Balance	Assimilation	
	Grain	Hay	Yield	Calcium	Phosphorus											
Basal ration (4 weeks):	*Kg*	*Kg*	*Kg*	*Pct.*	*Pct.*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Pct.*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Pct.*	
Cow A-37	66.5	65.3	143.1	0.121	0.111	874.4	172.8	1,063.7	+16.5	189.3	17.8	433.2	159.2	586.3	−6.1	153.1	26.1	1.81
Cow A-40	60.8	51.0	114.5	.125	.107	742.3	142.8	843.2	−41.9	100.9	12.0	433.0	122.5	520.2	−35.3	87.2	16.8	1.62
Cow 265	66.6	50.4	172.5	.104	.098	700.7	179.6	822.4	−57.9	121.7	14.8	429.0	166.0	549.7	−45.3	120.7	21.9	1.50
Alfalfa, timothy, and grain (4 weeks):																		
Cow A-37	70.0	70.0	135.7	.115	.116	595.5	156.7	727.0	−25.2	131.5	18.1	452.4	157.3	575.5	−34.2	123.2	21.4	1.26
Cow A-40	63.0	55.0	122.1	.123	.109	504.2	150.8	587.2	−67.8	83.0	14.1	417.7	133.4	505.5	−45.6	87.8	17.4	1.16
Cow 265	70.0	47.3	151.1	.099	.094	469.5	149.6	533.1	−86.0	63.6	11.9	468.5	142.8	540.0	−71.3	71.4	13.2	.99
Basal ration (2 weeks):																		
Cow A-37	70.0	70.0	130.7	.119	.115	966.3	156.2	1,127.5	+5.0	161.2	14.3	472.7	151.0	626.0	+2.3	153.3	24.5	1.80
Cow A-40	63.0	54.0	117.3	.127	.106	756.3	148.9	880.8	−24.4	124.5	14.1	428.8	124.9	548.4	−5.3	119.6	21.8	1.61
Cow 265	70.0	42.8	143.4	.101	.092	594.2	144.8	697.9	−41.1	103.7	14.9	451.1	132.7	560.8	−23.0	109.8	19.6	1.24
Basal ration, plus orthophosphoric acid (3 weeks):																		
Cow A-37	70.0	63.0	126.2	.120	.118	894.3	151.8	1,018.2	−27.9	123.9	12.2	662.8	148.8	795.3	−16.3	132.6	16.7	1.28
Cow A-40	63.0	56.0	115.9	.126	.110	767.1	145.6	905.6	−7.1	138.5	15.3	612.0	127.1	733.7	−5.4	121.7	16.6	1.23
Cow 265	70.0	49.5	153.9	.105	.100	685.0	161.5	818.9	−27.6	133.9	16.4	641.4	153.7	769.8	−25.3	128.4	16.7	1.06
Basal ration, plus monosodium phosphate (3 weeks):																		
Cow A-37	70.0	63.0	115.4	.118	.118	872.2	136.2	1,016.1	+7.7	143.9	14.2	640.8	135.8	799.7	+23.1	159.0	19.9	1.27
Cow A-40	63.0	56.0	110.2	.136	.110	728.0	150.2	904.2	+26.0	176.2	19.5	580.8	121.6	718.5	+16.1	137.6	19.2	1.26
Cow 265	70.0	63.0	173.5	.106	.099	808.2	183.3	1,016.1	+24.6	207.9	20.5	617.4	171.1	799.7	+11.2	182.3	22.8	1.27
Basal ration, plus disodium phosphate (3 weeks):																		
Cow A-37	61.7	63.0	97.5	.119	.116	861.2	115.6	1,011.6	+34.8	150.4	14.9	566.9	112.7	687.8	+8.2	120.9	17.6	1.47
Cow A-40	60.0	56.0	102.8	.139	.111	730.9	143.3	905.4	+31.2	174.5	19.3	530.3	114.5	658.1	+13.3	127.8	19.4	1.38
Cow 265	68.3	61.5	166.6	.106	.097	779.4	176.4	975.7	+19.9	196.4	20.1	575.5	161.1	735.9	−.7	160.4	21.8	1.33
Basal ration, plus trisodium phosphate (3 weeks):																		
Cow A-37	61.5	57.3	94.4	.114	.115	799.7	107.6	932.7	+25.4	132.9	14.3	561.5	108 8	657.8	−12.5	96.4	14.6	1.42
Cow A-40	53.3	50.3	87.9	.142	.109	643.7	124.7	818.1	+49.7	174.4	21.3	472.8	96.1	571.8	+2.9	99.1	17.3	1.43
Cow 265	70.0	63.0	170.3	.103	.097	848.8	176.0	1,027.7	+2.9	178.9	17.4	586.7	164.6	744.6	−6.7	157.9	21.2	1.38
Basal ration, plus orthophosphoric acid (3 weeks):																		
Cow A-37	63.0	56.0	83.0	.120	.117	773.5	99.3	912.0	+39.2	138.5	15.2	651.2	97.1	718.0	−30.3	66.9	9.3	1.27
Cow A-40	53.3	46.6	65.2	.154	.110	609.4	100.2	761.4	+51.8	152.0	20.0	551.0	71.7	624.1	+1.4	73.1	11.7	1.22
Cow 265	68.3	63.0	160.4	.104	.094	868.5	166.9	1,022.7	−12.7	154.2	15.1	655.9	150.9	764.0	−42.8	108.1	14.1	1.34

While this experiment was undertaken for the purpose of investigating the effect of variations in the alkalinity of the ration on the calcium and phosphorus metabolism of cows, yet, as the experiment progressed, other factors were indicated as of equal, or possibly greater, importance than the reaction of the ration, namely, the quantity and proportion of calcium and phosphorus in the ration.

A basal ration of alfalfa hay and grain probably does not contain the optimum proportion of phosphorus to calcium for high milk production, even though the grain (containing 30 percent wheat bran) has a fairly high phosphorus content. This is indicated by the fact that all the cows showed excessive losses of phosphorus from the body while on this ration during the first period. Insufficient phosphorus intake and rather generous milk flow are probably the factors causing the negative balances at this time. The calcium-phosphorus ratio in this period ranged from 1.5 to 1.8.

In the second period the intake of calcium was greatly reduced by the substitution of timothy hay for half of the alfalfa in the basal ration; while, in the third period, the calcium intake was increased by a return to the basal ration. The calcium and phosphorus balances became more negative in the second period, and less negative in the third period, but it is doubtful whether these changes in the balances have any physiological significance. The matter can be more profitably discussed in connection with some of the results of the second experiment, which will be given later.

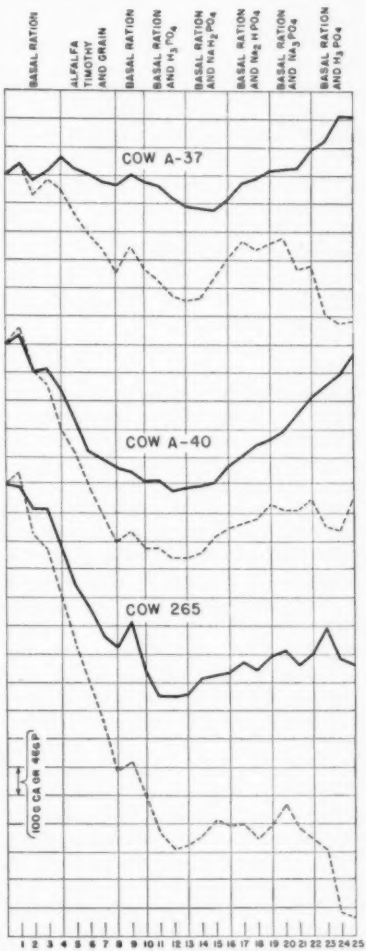
BASAL RATION
ALFALFA TIMOTHY AND GRAIN
BASAL RATION
BASAL RATION AND H_3PO_4
BASAL RATION AND NaH_2PO_4
BASAL RATION AND Na_2HPO_4
BASAL RATION AND Na_3PO_4
BASAL RATION AND H_3PO_4
COW A-37
COW A-40
COW 265
100 G CA OR 46 G P
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
WEEKS

FIGURE 1.—Fluctuation in the content of calcium and phosphorus in the bodies of the cows during the course of the first experiment. The solid line represents calcium; the dotted line, phosphorus. The divisions on the ordinate correspond to 100 grams of calcium or 46 grams of phosphorus, the ratio in which the elements are present in bone.

With the introduction of the phosphorus supplements during the remainder of the experiment the phosphorus intake was materially increased. This increased phosphorus intake did not appear to be very effective in preventing mineral losses, however, when supplied in the form of orthophosphoric acid, as in the fourth and eighth periods. The quantities of

phosphorus in the ration and the calcium-phosphorus ratio assumed more appropriate values (average 1.19). In spite of this the animals continued to lose calcium and phosphorus in the fourth period. In the eighth period, when the same supplement was fed, the phosphorus losses were considerable in the case of cows A–37 and 265. Hart (*1*) recently observed, after administering hydrochloric acid to cows, that there was a divergence of the calcium excretion from the feces to the urine but no improvement in calcium balances.

During the fifth and sixth periods, monosodium and disodium phosphates were used as supplements and the effect was marked. Not only were calcium and phosphorus losses checked in the case of all animals but a distinct recovery of mineral stores was initiated which extended even into the seventh period. This occurred without any great reduction in milk yield and while the calcium-phosphorus ratio was being maintained at the above-mentioned appropriate value.

The effect of trisodium phosphate as a supplement was somewhat irregular. Possibly the alkalinity of this material was unfavorable for mineral absorption.

These results would seem to indicate that by merely supplying a suitable neutral phosphorus supplement in sufficient quantity and in proper ratio to the calcium present in the ration (that calcium being already present in an available form and in generous amount), a phosphorus-deficient ration can be made adequate and equilibrium or positive balances can be obtained. It seems reasonable to assume, since the mature high-producing cow's chief need for calcium and phosphorus is to secrete them in the milk, that the ration which she receives should contain calcium and phosphorus in approximately the same proportion that they occur in milk, namely, about 1.1:1. If calcium and phosphorus are supplied in assimilable form and in sufficient quantity and in the proportion present in milk (the ration being satisfactory in other known respects), then, the writers believe, much will have been done to improve the mineral nutrition of the cow.

Phosphorus, in the form of disodium phosphate, was fed to dry cows at this station several years ago (*4*). Very definite increases in milk yield during the subsequent lactations were noted. At that time, however, only a few short-time balances were followed where the cows were receiving a mineral phosphorus supplement.

Since the supplements monosodium and disodium phosphate have brought about so marked a retention of calcium and phosphorus in animals that have suffered considerable mineral losses, they should also be effective in preventing such losses in animals in a better state of nutrition. That such losses, however, are not entirely preventable is evident from an experiment conducted at this station recently (*7*). In this instance two cows giving 21 to 28 kilograms of milk daily were fed the best ration that the writers could devise, including a supplement of disodium phosphate. With average daily calcium and phosphorus intakes of 129 and 117 grams, respectively, these cows were slowly but steadily losing calcium and phosphorus from their bodies. It seems impossible to escape the conclusion recently stated by Hart (*1*), that "in the early period of lactation, *especially with high milk flow*, the calcium assimilation from the digestive tract is insufficient to meet the needs of mammary secretion and

the skeleton is drawn upon, with a negative calcium balance resulting." The interdependence of calcium and phosphorus metabolism involves a simultaneously lowered phosphorus assimilation.

SECOND EXPERIMENT: BONE MEAL AS A PHOSPHORUS SUPPLEMENT

A second experiment, conducted in a somewhat different manner, was completed about a year later. In this experiment bone meal was used as a supplement instead of the soluble phosphates used in the first experiment. Bone meal is so often employed as a source of calcium and phosphorus for cattle that it was thought advisable to study its effects on the metabolism of these elements; but, as will appear later, its use introduces experimental complications, which make it necessary to exercise great caution in drawing conclusions as to the physiological significance of the results.

EXPERIMENTAL PROCEDURE

Three grade Holstein cows were used in this experiment—cows A–37, A–43, and A–46. Cows A–37 and A–46 were not pregnant; cow A–43 had been pregnant for about a month at the end of the experiment. The animals were from 3½ to 5 years of age.

The experiment began November 5 and ended January 13, a period of 10 weeks. During the first 3 weeks of the experiment the same basal ration was used as in the first experiment. During the next 7 weeks supplements of bone meal and disodium phosphate were added to the basal ration, bone meal the first 3 weeks and phosphate the last 4 weeks. The amounts used were so regulated as to introduce a uniform increase in the phosphorus intake, that is, one which, when introduced in the form of sodium phosphate, would keep the calcium-phosphorus ratio in the feed between 1.1 and 1.5. Obviously when bone meal was used it was impossible to make this correction in the calcium-phosphorus ratio of the feed. These supplements were added to the grain mixture, and in the case of bone meal, represented about 3.8 percent, and in the case of the disodium phosphate, about 6.5 percent of the mixture.

The weights of the cows at the beginning and end of the experiment were respectively: Cow A–37, 568 and 575 kilograms; cow A–43, 439 and 469 kilograms; cow A–46, 452 and 461 kilograms.

Hay and grain were fed twice a day and the cows were milked three times a day.

The alfalfa hay contained about 1.5 percent calcium and 0.2 percent phosphorus, the grain 0.13 percent calcium and 0.7 percent phosphorus. Chemically pure disodium phosphate and a high grade of bone meal were used to supplement the grain mixture and were thoroughly mixed with it. The bone meal contained 29.45 percent calcium and 14.06 percent phosphorus.

EXPERIMENTAL RESULTS

The milks secreted showed a uniform slight increase in phosphorus content when bone meal was fed (table 2).

TABLE 2.—*Weekly feed consumption, milk yield, percentage of calcium and phosphorus in the milk, and the calcium and phosphorus balances for the 3 cows during the different feeding periods*

COW A-37

Ration and period	Feed consumed		Milk produced			Calcium					Phosphorus					Calcium-phosphorus ratio in feed
			Yield	Composition		In urine and feces	In milk	In feed	Balance	Assimilation	In urine and feces	In milk	In feed	Balance	Assimilation	
	Grain	Hay		Calcium	Phosphorus											
	Kg	*Kg*	*Kg*	*Pct.*	*Pct.*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams* *Pct.*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams* *Pct.*	
Basal ration:																
First week	77	77	155.9	0.124	0.119	1,052.3	193.3	1,349.7	+104.1	297.4 22.0	479.7	185.5	679.6	+14.4	199.9 29.4	2.0
Second week	77	77	153.0	.124	.116	1,050.0	189.7	1,352.8	+113.1	302.8 22.4	504.8	177.5	697.9	+15.6	193.1 27.7	2.0
Third week	77	77	149.4	.129	.118	1,130.3	192.7	1,351.9	+28.9	221.6 16.4	542.7	176.3	708.6	−10.4	165.9 23.4	2.0
Average	77	77	152.8	.126	.118	1,077.5	191.9	1,351.5	+82.0	273.9 20.3	509.1	179.8	695.4	+6.5	186.3 26.8	2.0
Basal ration, plus bone meal:																
Fourth week	77	77	149.5	.120	.121	1,884.1	179.4	2,184.1	+120.6	300.0 13.7	844.6	180.9	1,097.1	+71.6	252.5 23.0	2.0
Fifth week	77	77	142.9	.124	.120	1,880.2	177.2	2,158.1	+100.7	277.9 12.9	857.0	171.5	1,064.0	+35.5	207.0 19.5	2.0
Sixth week	60.5	66	132.4	.119	.119	1,529.7	157.6	1,722.6	+35.3	192.9 11.2	711.5	157.6	845.7	−23.4	134.2 15.9	2.0
Average	71.5	73.3	141.6	.121	.120	1,764.7	171.4	2,021.6	+85.5	256.9 12.6	804.4	170.0	1,002.3	+27.9	197.9 19.5	2.0
Basal ration, plus sodium phosphate:																
Seventh week	66	77	139.1	.117	.118	1,203.4	162.7	1,262.1	−104.1	58.7 4.7	816.8	164.1	949.4	−31.5	132.6 14.0	1.3
Eighth week	60.5	77	130.6	.117	.114	1,117.9	152.8	1,258.6	−12.1	140.7 11.2	715.4	148.9	868.5	+4.2	153.1 17.6	1.4
Ninth week	55	77	130.3	.119	.118	1,152.5	155.1	1,248.9	−58.7	96.4 7.7	750.8	153.8	838.5	−66.1	87.7 10.5	1.5
Tenth week	66	77	134.7	.119	.117	1,021.2	160.3	1,231.0	+49.5	209.8 17.0	794.3	157.6	944.7	−7.2	150.4 15.9	1.3
Average	61.9	77	133.7	.118	.117	1,123.8	157.7	1,250.2	−31.3	126.4 10.2	769.3	156.1	900.3	−25.2	131.0 14.5	1.4

COW A-43

Basal ration:																
First week	70	63	149.0	0.120	0.095	878.4	178.8	1,113.2	+56.0	234.8 21.1	457.4	141.6	603.5	+4.5	146.1 24.2	1.8
Second week	70	63	148.6	.121	.091	869.2	179.8	1,116.0	+67.0	246.8 22.1	463.0	135.2	610.1	+11.9	147.1 24.1	1.8
Third week	70	49.5	139.5	.122	.096	852.1	170.2	895.8	−126.5	43.7 4.9	501.5	133.9	602.2	−33.2	100.7 16.7	1.5
Average	70	58.5	145.7	.121	.094	866.6	176.3	1,041.7	−1.2	175.1 16.0	474.0	136.9	605.3	−5.6	131.3 21.7	1.7

Basal ration, plus bone meal:																	
Fourth week	70	58.5	145.5	.114	.098	1,539.4	165.9	1,823.6	+118.3	284.2	15.6	769.7	142.6	981.8	+69.5	212.1	21.6	1.9
Fifth week	70	63	150.1	.120	.098	1,625.7	180.1	1,854.7	+48.9	229.0	12.3	781.5	147.1	953.1	+24.5	171.6	18.0	1.9
Sixth week	70	63	143.6	.121	.100	1,588.3	173.8	1,787.1	+25.0	198.8	11.1	819.6	143.6	951.7	−11.5	132.1	13.9	1.9
Average	70	61.5	146.4	.118	.099	1,584.5	173.3	1,821.8	+64.1	237.3	13.0	790.3	144.4	962.2	+27.5	171.9	17.8	1.9
Basal ration, plus sodium phosphate:																		
Seventh week	70	63	141.5	.114	.100	940.2	161.3	1,052.7	−48.8	112.5	10.7	868.2	141.5	969.4	−40.3	101.2	10.4	1.1
Eighth week	70	63	146.1	.115	.099	927.9	168.0	1,056.0	−39.9	128.1	12.1	819.5	144.6	955.6	−8.5	136.1	14.2	1.1
Ninth week	70	63	145.3	.117	.098	895.6	170.0	1,052.9	−12.8	157.2	14.9	843.4	142.4	997.9	+12.1	154.5	15.5	1.1
Tenth week	70	63	149.2	.114	.100	907.5	170.1	1,052.8	−50.8	119.3	11.6	833.3	149.2	964.7	−17.8	131.4	13.6	1.1
Average	70	63	145.5	.115	.099	917.8	167.4	1 047.1	−38.1	129.3	12.3	841.1	144.4	971.9	−13.6	130.8	13.4	1.1

COW A-46

Basal ration:																	
First week	70	70	130.6	0.127	0.113	1,002.5	165.9	1,227.0	+58.6	224.5	18.3	477.2	147.6	617.8	−7.0	140.6	22.8	2.0
Second week	70	70	129.9	.124	.109	980.4	161.1	1,229.8	+88.3	249.4	20.3	479.5	141.6	634.4	+13.3	154.9	24.4	1.9
Third week	70	60	120.6	.126	.107	914.4	152.0	1,066.5	+.1	152.1	14.3	499.1	129.0	623.7	−4.4	124.6	20.0	1.7
Average	70	66.6	127.0	.126	.110	965.8	159.7	1,174.4	+49.0	208.7	17.6	485.3	139.4	625.3	+.6	140.0	22.4	1.9
Basal ration, plus bone meal:																		
Fourth week	70	60	124.4	.119	.115	1,589.6	148.0	1,848.5	+110.9	258.9	14.0	794.8	143.1	984.9	+47.0	190.1	19.3	1.9
Fifth week	70	70	126.4	.124	.113	1,715.4	156.7	1,961.9	+89.8	246.5	12.6	796.3	142.8	967.2	+28.1	170.9	17.7	2.0
Sixth week	70	70	122.1	.125	.115	1,721.3	152.6	1,894.3	+20.4	173.0	9.1	838.1	140.4	965.8	−12.7	127.7	13.2	2.0
Average	70	66.6	124.3	.123	.114	1,675.4	152.4	1,961.6	+73.7	226.1	11.9	809.7	142.1	972.6	+20.8	162.9	16.7	2.0
Basal ration, plus sodium phosphate:																		
Seventh week	65	70	117.6	.123	.116	1,071.1	144.6	1,153.6	−62.1	82.5	7.2	801.1	136.4	923.3	−14.2	122.2	13.2	1.2
Eighth week	65	70	116.7	.121	.114	1,016.5	141.2	1,157.0	−.7	140.5	12.1	814.0	133.0	909.1	−37.9	95.1	10.5	1.3
Ninth week	65	70	106.4	.122	.109	1,031.8	129.8	1,154.0	−7.6	122.2	10.6	776.1	116.0	948.1	+56.0	172.0	18.1	1.2
Tenth week	60	70	113.7	.120	.113	1,001.1	136.4	1,119.2	−18.3	118.1	10.6	798.2	128.5	855.2	−71.5	57.0	6.7	1.3
Average	63.7	70	113.6	.122	.113	1,030.1	138.0	1,146.0	−22.2	115.8	10.1	797.4	128.5	908.9	−16.9	111.6	12.1	1.3

Figure 2 shows the fluctuation in calcium and phosphorus content in the bodies of the cows during the course of the experiment.

From a study of table 2 and figure 2 it is evident that the cows retained calcium during the period in which they were on the basal ration (except cow A–43) and that they retained even more calcium as well as phosphorus during the bone-meal supplement period but lost both calcium and phosphorus during the sodium-phosphate supplement period.

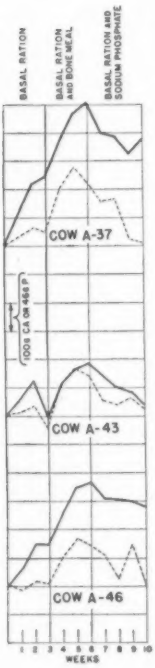

FIGURE 2.—Fluctuation in the content of calcium and phosphorus in the bodies of the cows during the course of the second experiment. The solid line represents calcium; the dotted line, phosphorus. The divisions on the ordinate correspond to 100 grams of calcium or 46 grams of phosphorus, the ratio in which these elements are present in bone.

The effects of changes in rations on calcium and phosphorus balances noted here are typical of those observed in all balance experiments where the intakes of calcium and phosphorus are varied markedly during the experiment. It is probable, however, that these changes do not represent alterations in the amounts of calcium and phosphorus stored in the bones or other internal tissues of the animal, but changes primarily in the amounts of calcium and phosphorus held in mechanical suspension in the fluid contents of the stomachs and intestines.

The bovine intestinal tract has a very complicated form, consisting of the four stomachs, one of which has highly corrugated walls, in addition to the large intestine and the very long and convoluted small intestine. There can be no doubt that considerable proportions of such insoluble material as tricalcium phosphate, or of any calcium and phosphorus compounds capable of forming tricalcium phosphate, when introduced into this tract with the food, are likely to remain in it for long periods. Furthermore, the normal amount of the bovine intestinal contents or "fill" is very large. In the case of three cows of the Jersey type recently slaughtered at this station after they had fasted for 24 hours, the average amount of fill was 52 kilograms. It is probable that in the case of Holstein cows on full feed the fill would be about 100 kilograms.

Some idea as to how much calcium is likely to be retained in the intestinal contents and how long this element is likely to go on increasing there during a period of bone-meal feeding may be obtained from the figures given for the calcium in the urine and feces of cows A–43 and A–46 in table 2. Cow A–37 is omitted from this discussion because she was badly off feed in the last of the periods in which bone meal was fed. It will be seen that the calcium content of the urine and feces of cows A–43 and A–46 did not reach its height until the second or third week of bone-meal feeding.

Unfortunately the percentage of calcium in the feces alone was not determined. However, the percentage of calcium in the amount of feces and urine discharged weekly and mixed with a small amount of wash water was determined; and, as the urine contains very little calcium as compared with the feces, these figures give a rough idea of

the amounts of calcium contained in the intestinal contents at the beginning and end of the bone-meal feeding period. The actual amount of calcium contained in the fill at the beginning of this period, and the actual increase during its progress would, of course, be considerably larger than these figures indicate.

The average amount of calcium in the urine and feces of cows A–43 and A–46 during the last period that they were on the basal ration was 0.238 percent, and during the last period on bone meal it was 0.378 percent. On the supposition that the fill amounted in each case to 100 kilograms, this would mean that the intestinal contents of the cows had 238 grams of calcium at the beginning of the bone-meal feeding period and 378 grams at the end, and that the calcium retained in the intestinal tract increased by 140 grams during this period. This increase accounts for most of the increase in body calcium as shown by the balance results. It is not unreasonable to suppose, therefore, that the positive calcium and phosphorus balances in the second period of the experiment represent merely an accumulation of these elements in the intestinal contents; and that the negative balances in the third period represent a gradual loss of this accumulation after the daily intake had been decreased.

It is not unlikely that a somewhat similar explanation may account for the changes in the balances which took place in the first, second, and third periods of the first experiment where the intake of calcium was reduced by the feeding of timothy hay. This interpretation would not appear to invalidate the conclusions expressed in respect to the different phosphate supplement periods where the intakes of calcium and phosphorus were maintained quite uniformly constant.

SUMMARY AND CONCLUSION

Two experiments made to determine the relative value of certain soluble phosphates and bone meal as phosphorus supplements in dairy rations, are reported. In the first experiment the phosphorus supplements were orthophosphoric acid, monosodium, disodium, and trisodium phosphates; in the second experiment bone meal was used. During the first period of each experiment the same basal ration was fed, but the first experiment was started in late September, when hot weather and annoyance from flies reduced the consumption of feed, whereas the second experiment was started in the cooler weather of November. The result was smaller intakes of calcium and phosphorus and negative balances in the first experiment and larger intakes of calcium and phosphorus and positive balances in the second experiment.

Dairy rations of alfalfa hay and grain often have a rather large proportion of calcium as compared with phosphorus. The experiments here reported indicated that calcium and phosphorus balances of cows fed on such rations may sometimes be rendered more positive by adding orthophosphates to them. This improvement is more marked when the nearly neutral phosphates disodium phosphate, and monosodium phosphate are used than when orthophosphoric acid or trisodium phosphate is used.

Large increases in the amount of calcium and phosphorus received by cows in their rations are likely to be followed by more positive calcium and phosphorus balances; decreases, by less positive balances.

There is reason to believe, however, that considerable quantities of calcium and phosphorus may be retained as insoluble tricalcium phosphate for several weeks in the intestinal tracts of cows, and there is no way of knowing what proportion of positive calcium and phosphorus balances is to be explained in this way and what proportion really represents a gain in bone tissue. Changes in the balances, following large changes in the calcium and phosphorus intake, and lasting not more than a few weeks, should not therefore be taken as any certain indication of changes in the assimilation of calcium and phosphorus from the intestinal tract.

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INHERITANCE OF RESISTANCE TO LOOSE SMUT IN CERTAIN WHEAT CROSSES[1]

By D. C. TINGEY, *assistant agronomist*, and BION TOLMAN, *graduate student, Utah Agricultural Experiment Station*[2]

INTRODUCTION

During recent years the principles of Mendelism have been applied extensively in the production of new types of plants possessing resistance to various diseases. The results of this mode of attacking the disease problem have been highly favorable. Old varieties are gradually giving way to newer types equal to or exceeding the old in quality and productivity as well as possessing resistance to one or more diseases.

Loose smut in wheat, *Ustilago tritici* (Pers.) Jens., while not as serious a problem in Utah as the covered smut (*Tilletia* spp.) has,

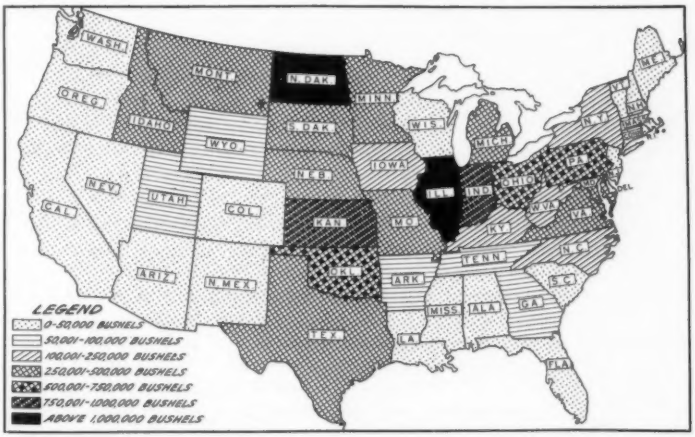

FIGURE 1.—Estimated average annual loss of wheat due to loose smut, by States, 1917–26, inclusive. From Journal of Agricultural Research 39: 314 (1929).

according to Tapke (*16*)[3] caused an average annual loss in this State of between 50,000 and 100,000 bushels of wheat (fig. 1). The various methods advocated for the control of loose smut in wheat, with the exception of the use of resistant varieties and hot-water treatments, have been either impractical of application or ineffective in control, or both (*16*).

[1] Received for publication, Sept. 26, 1933; issued June, 1934.

[2] The results reported herein were obtained in cooperation with the Division of Creal Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture. The writers are indebted to R. J. Evans, agronomist and A. L. Wilson, associate horticulturist, of the Utah Agricultural Experiment Station; to V. H. Tingey, assistant professor of mathematics, of the Utah State Agricultural College; and to F. A. Abegg, associate geneticist, Division of Sugar Plant Investigations, U.S. Department of Agriculture, who critically read the manuscript. Acknowledgment is made to V. F. Tapke for the use of figures 1 and 2. The writers wish also to express their appreciation to Margaret Richards Cook, who calculated the data in the tables.

[3] Reference is made by number (italic) to Literature Cited, p. 655.

According to Tapke (*16*) the modified hot-water treatment devised by Freeman and Johnson (*4*) has generally been the method recommended. While this method is effective, if properly carried out, it is rather complicated and tedious to apply, especially for farmers, who usually are not properly equipped. Because of this and the fact that the disease frequently escapes observation, seed treatment for the control of loose smut is seldom practiced; as a result, the disease is allowed to go unchecked. The development of a resistant variety possessing the other desirable characteristics of locally grown spring wheats would be a decided advantage to the farmers in combating the disease.

REVIEW OF LITERATURE

A number of studies have been made on the comparative resistance of wheat varieties to loose smut. Tapke (*16*) gives an account of his studies on varietal resistance to this disease. It is evident from his work, as well as that of others, that some varieties exhibit greater resistance than others. Matsuura (*12*) reviews a report, Washington Agricultural Experiment Station Bulletin 155, presumably on the inheritance of resistance to *Ustilago tritici*. However, the original article refers only to smut and does not state whether it was *U. tritici* or *Tilletia tritici*. An article by T. Kilduff (*9*) came to the attention of the writers as this manuscript was ready for publication. The writer was unable to give a genetic analysis of inheritance to loose-smut resistance.

EXPERIMENTAL MATERIAL AND METHODS

PARENTAL MATERIAL USED

Varieties and strains of wheat used in these studies were Hope C.I. 8178; Preston C.I. 3081; 01–24, C.I. 11542; Dicklow No. 3; and Federation. These are all classed as *Triticum vulgare* Vill. wheats. The two leading spring varieties grown in Utah are Federation and Dicklow. Dicklow No. 3 is a Utah selection out of the Dicklow variety. This strain is the one used as a check in the wheat-nursery tests conducted at the station. It is more uniform, is less subject to lodging, and is a slightly higher yielder than the ordinary Dicklow variety. Strain 01–24 is a new production from the Utah station. It is a strong-strawed, high-yielding, white-kerneled spring wheat. The parents of this strain are not definitely known. However, it is probably a segregate out of either a Dicklow × Federation or a Dicklow × C.I. 4722 cross. The reaction of this strain to loose smut places some doubt on the possibility of its being out of the Dicklow × Federation cross, as Federation appears to possess no factors for resistance and 01–24 is more resistant than the Dicklow No. 3 strain. There is, however, the possibility that the Dicklow or Federation used in the cross from which 01–24 might have been selected was more resistant than the Dicklow No. 3 or the Federation used in these studies. Selections of Dicklow and Federation made at the Utah station show definitely that these two varieties do possess individuals differing in physiological characters; similar or even greater differences are possible in regard to their reaction to loose smut. A history of the origin and development of Hope C.I. 8178 is given by McFadden (*10*); Preston C.I. 3081 and Federation are described by Clark et al.

(*1*). The varieties, with contrasted characters studied, are shown in table 1.

TABLE 1.—*Contrasted characters of the parents studied in the crosses*

Parental variety	Morphological characters			Average percentage of infection with loose smut
	Presence or absence of awns	Chaff color	Grain color	
Hope	Fully awned	White	Red	0
Preston	do	do	do	1.6±0.39
01-24	Short apical awns	Bronze	White	18.55±1.21
Dicklow No. 3	do	White	do	38.47±1.27
Federation	Short beaks	Bronze	do	73.50±1.57

Inoculation experiments conducted in 1928 showed Hope wheat to be completely immune to the inoculum used. Later McFadden (*10*), who is responsible for the development of this variety, was led to conclude from his observations and tests that Hope wheat is highly resistant, if not immune, to the loose smut occurring on Kota. Other strains and varieties were found to possess varying degrees of resistance. However, Dicklow and Federation were found to be susceptible to the inoculum used. Federation was highly susceptible, whereas Dicklow possessed only a fair degree of resistance. This was somewhat surprising as Dicklow has consistently smutted under natural conditions more than Federation. However, it is undoubtedly due to a type of morphological resistance possessed by the Federation and not by the Dicklow variety. This resistance appears to be of such a nature as to prevent the smut spore from coming in contact with the stigma, such as having closer flowering glumes or having stigmas which are less likely to protrude outside the flowering glume during the blooming period.

INOCULUM USED

One of the difficulties in attempting to place the inheritance of disease resistance on a definite factorial basis is the possibility that the inoculum used may not be of a single physiologic form.

Rodenheiser (*14*) concluded from culture studies on nutrient media that there were physiologic forms of *Ustilago tritici* and *U. nuda* (Jens.) K. and S. In fact, he is of the opinion that *U. tritici* and *U. nuda* are physiologic forms rather than separate species. Whether these or other forms will be different pathogenically remains to be determined. However, there is reason to believe that there are different physiologic forms of *U. tritici*. Humphrey and Tapke (*8*) conclude from cross-inoculation experiments that wheat and rye smuts were identical *U. tritici*. Reed (*13*) reports physiologic strains within *U. avenae* (Pers.) Jens. Faris (*2*) has reported similar results on *U. hordei* (Pers.) K. and S. Tisdale and Johnson (*17*) and Stakman and Christensen (*15*) have demonstrated the existence of physiologic strains of *U. zeae* (Beckm.) Ung. The inoculum used was originally taken from the Dicklow variety, and it apparently spread to Federation and Sevier. Dicklow has been grown at the Utah station for years, and the other varieties, except Federation and Sevier, have shown little or no infection. Some preliminary data secured seem to indicate that the inoculum used in these studies was

comparatively uniform pathogenically. In figure 2 are shown two spikes of wheat, the one healthy and the other infected with loose smut.

EXPERIMENTAL METHODS

Pure-line crosses between Hope × Federation, Hope × Dicklow No. 3, and Preston × 01–24 were made at the Central Experimental Farm (North Logan) in 1928. Pollen from anthers of a single spike was used to pollinate the stigmas of a single spike. The progeny of a single F_1 plant were seeded in the spring of 1929. The kernels were spaced about 2.5 inches apart in a row. Inoculations were made by the time the spikes reached the full bloom stage. In preparing the heads for inoculation, the center and the basal and terminal florets of each spikelet were removed; if awns were present, they were clipped off. The glumes of the remaining florets were then spread apart with small hand forceps, and the stigma was thoroughly dusted with the inoculum. Seedlings were made from each F_2 plant. The number of F_2 plants represented in F_3 rows are shown in the various goodness-of-fit tables. The F_3 rows were sown in randomized blocks with duplicate plantings of the Hope × Federation cross and three replicates of the Hope × Dicklow No. 3 and Preston × 01–24 crosses. A number of parental rows were sown at random over the experimental area. The genetic characters studied were resistant to loose smut, awns, glume, and kernel color. Smut-infection data were based on plant count and not on head count.

FIGURE 2.—*A*, Healthy spike of wheat; *B*, smutted spike. (From Journal of Agricultural Research, 39: 316 (1929).

In studying the goodness of fit, the χ^2 test, as given by Fisher (*3*), was used.

EXPERIMENTAL RESULTS

PRELIMINARY EXPERIMENTS ON INOCULATION TECHNIC

Some preliminary studies were made in 1928 in order to determine the proper time and method of inoculation to insure maximum infection.

METHODS OF INOCULATION

Chlamydospores were suspended in water and then placed on the stigmas of some of the plants; the powdered inoculum was placed on

the stigmas of others by opening the glumes and applying the spores with a pair of forceps. The third method tried was that of heavily dusting the dry spores on the spikes of plants; and the fourth method was that of dipping the spike in a beaker of water heavily laden with spores of loose smut. Table 2 gives the results of this test.

TABLE 2.—*Percentage of infection obtained from various methods of inoculation (given to the nearest whole percent)* [a]

Method of inoculating	Federation		Dicklow No. 3	
	Total plants	Plants infected	Total plants	Plants infected
	Number	*Percent*	*Number*	*Percent*
Spores placed on stigma:				
Dusted on	73	60	54	52
Suspended in water	21	50	11	57
Spores placed on the spikes:				
Dusted on	59	15	72	34
Suspended in water	22	5	12	0
Check (no inoculation)	17	0	15	0

[a] Data in this table show little difference in the percentage of infection in Dicklow No. 3 and in Federation. The percentage of infection is somewhat high for one and correspondingly low for the other as compared with data in table 1. This is probably due to the fact that the data here are based on single 6-foot rows subject to rather wide variations, whereas data in table 1 represent an average for a number of rows.

It is evident from table 2 that the most effective methods of inoculating were those which involved the placing of the spores directly on the stigmas. However, there was no difference between placing the spores on the stigmas when dry or suspended in water. Dusting the spikes with the spores appeared more effective than dipping them in water containing spores. It will be seen from table 3 (compare with table 4), however, that response to dusting spikes with the inoculum varied with the variety and, therefore, could not be relied upon to give satisfactory results in genetic studies.

TABLE 3.—*Reaction of the wheat varieties and strains to loose smut when the inoculum was dusted on the spikes*

Wheat variety or strain	Total plants	Plants infected	Infection in check plants [a]	Wheat variety or strain	Total plants	Plants infected	Infection in check plants [a]
	Number	*Percent*	*Percent*		*Number*	*Percent*	*Percent*
Dicklow	72	34.7	0.0	Q-250	105	1.9	2.4
Dicklow No. 3	53	34.0	6.1	R-18-5	77	10.4	.0
Dicklow No. 16	61	41.0	.0	R-48-22	82	2.4	.0
Federation	59	15.2	.0	R-S 4-5	106	7.4	.0
Hard Federation	60	.0	.0	R-S 17	72	4.1	.0
Marquis	54	3.6	.0	A-4	70	.0	.0
Alcalde	58	.0	.0	14-85	81	1.2	.0
Onas	52	13.0	.0	G-40	60	.0	.0
Sevier A	37	27.0	.0	G-43-11	75	4.0	.0
Sevier 59	36	16.7	.0	G-48	62	6.4	.0
Sevier 125	31	6.5	.0	G-149	65	3.1	.0
No. 139-3	59	11.9	.0	4-287	67	3.0	.0
No. 146	57	7.0	1.2	5-185	68	7.3	1.0
No. 49-10	43	4.7	.0	9-7	48	10.5	.0
No. 1-174-2	68	10.3	1.6	11-12	80	13.7	.0
01-24	47	10.6	.0	11-88	74	1.5	.0
Q-80	91	6.6	.18	12-101	65	.0	.0
Q-227	80	3.7	.0	13-47	83	3.5	.0
Q-231	68	1.4	.0				

[a] Checks not inoculated.

Time of Inoculation

Inoculations were made at three different stages of anthesis: (1) When the stamens were green, (2) when they were yellow, and (3) when the pollen was being shed. In all cases the smut was placed directly on the stigma with forceps. Data on the time of inoculation are given in table 4. Student's pairing method (*3*) was used to determine whether there were any significant differences. It is obvious from the probability, as shown by P in these tables, that there are no significant differences; at least, if there are any, they are covered up by the differential reaction of the varieties.

TABLE 4.—*Percentage of loose-smut infection obtained on different varieties and strains inoculated at two different stages of anthesis and the probability of a difference in the two means*

GREEN AND YELLOW STAMENS

Wheat variety or strain	Percentage of infection when plants were inoculated while stamens were—		Wheat variety or strain	Percentage of infection when plants were inoculated while stamens were—	
	Green	Yellow		Green	Yellow
Alcalde	100.0	100.0	R–S 4–5	77.8	76.9
Onoas	95.0	66.7	R–S–17	77.8	100.0
Sevier 125	60.0	80.0	11–30	70.0	[a]80.0
1–46	73.7	90.5	Dicklow No. 3	83.3	64.4
Q–248	50.0	60.0	Federation	80.0	[b]79.80
II–R–18–5	80.0	88.9			
R–48–22	63.6	60.6	Mean	75.9	79.0

STAMENS GREEN AND STAMENS SHEDDING POLLEN

	Green	Shedding pollen		Green	Shedding pollen
Marquis	33.3	33.3	Dicklow No. 3	83.3	[c]50.0
Q–227	81.8	30.8	Federation	80.0	[d]89.6
9–7	87.5	78.6			
14–61	22.2	31.6	Mean	64.7	52.3

STAMENS YELLOW AND STAMENS SHEDDING POLLEN

	Yellow	Shedding pollen		Yellow	Shedding pollen
01–24	81.2	81.2	Dicklow No. 3	64.4	[e]50.0
Q–89	90.9	65.0	Federation	79.8	[f]89.6
F–68	36.4	52.9			
11–12	81.8	25.0	Mean	73.1	66.2
11–88	76.9	100.0			

[a] $t=0.71$. [b] $P=0.5$. [c] $t=1.23$. [d] $P=0.2$–0.3. [e] $t=0.65$. [f] $P=0.5$–0.6.

The data presented in table 4 at first seemed to indicate that more infection was obtained when the stamens were green, as was stated by Tapke (*16*). However, when the data were analyzed statistically, no significant difference was noted between the three periods of inocula-

tion. Maddox (*11*) states that the time of maximum infection is during the period when the pollen is being shed. Freeman and Johnson (*4*) concluded that maximum infection occurs during the period of full bloom and that some degree of infection occurs until the ovary has reached one third its mature size.

STUDIES ON INHERITANCE OF RESISTANCE TO LOOSE SMUT

Difficulties in Placing Resistance on a Definite Factorial Basis

Several difficulties are encountered in attempting to place on a definite factorial basis the inheritance of resistance to loose smut, as well as to any other disease. One of the most complicating factors is the effect of environment. This effect was partly reduced by replication. Some comparatively susceptible types occasionally escape the disease when grown in short rows, even though artificially inoculated. For example, Preston on the average smuts about 1.6 percent, with some rows smutting as high as 9.5 percent; however, 70 percent of the rows, in these studies with 30 seeds sown in each row, entirely escaped infection.

The possibility of the inoculum not being entirely uniform, because of the possible existence of physiologic forms of loose smut, also adds to the difficulty of placing the inheritance of resistance on a definite factorial basis. In spite of these complications, an attempt has been made to place resistance on a Mendelian basis.

Relation of Infection to Sheath Color

An interesting condition developed on the sheaths and the exposed culms of inoculated plants. Usually the plants with smutty spikes developed a distinct grayish-purple color on the leaf sheaths. At first it appeared as if the coloration were a characteristic of the culm; on closer examination it was found that the coloration was generally confined to the sheath portion of the leaf. It was also found on the portion of the culms exposed to the light. This peculiar coloration developed on Hope and Preston during the year they were inoculated, whereas on Federation no color developed even though the plants smutted. In the F_3 generation there appeared to be a segregation for this condition, suggesting that it may partly be controlled by genetic factors. According to Heald (*7, pp. 683–684*), a similar condition was observed by McAlpine, who stated that "when a stool is affected with loose smut, the stalks are generally of a purplish tint, so that they can be readily picked out from among the general crop."

Biometrical Studies

RELATION OF SMUT INFECTION AND SEEDLING MORTALITY

In the study of disease resistance where the disease organism is operative during the seedling stage of the host plant, it is important to know whether or not there is any differential relationship between the infection and seedling mortality among resistant and susceptible lines. If such a relationship existed, it would no doubt materially complicate a genetic interpretation of inheritance. In order to determine whether this condition did exist, a known number of kernels were seeded in each F_3 row. This made it possible to calculate the

percentage of seedling plants reaching maturity, or the percentage stand. It appeared evident that if the disease were causing the death of any appreciable number of susceptible seedlings, there should be a relationship in the percentage of smut obtained in the F_3 rows and the percentage stand. Simple correlation coefficients were used to measure whether or not a relationship existed; since F_3 rows were replicated, an average of the replication was taken for both the percentage of smut and the percentage of stand; this average replication was used in calculating the correlations. Correlation coefficients thus obtained, between the percentage of smut and the percentage of stand for the susceptible parental strain and for the F_3-progeny rows, are shown in the following tabulation:

Material:	r
Federation	0. 09 ± 0. 167
Dicklow No. 3	. 10 ± . 115
01–24	. 16 ± . 096
Hope × Federation (F_3 rows)	. 02 ± . 047
Hope × Dicklow No. 3 (F_3 rows)	. 02 ± . 039
Preston × 01–24 (F_3 rows)	. 01 ± . 039

There seems to be no evidence from these data that smut has any differential influence on the percentage stand in the F_3 rows of resistant and susceptible lines. This is further shown in considering the average percentage stand obtained in the F_3 parental rows, since here it is possible to compare the inoculated resistant and susceptible strains. The average percentage stand was: Hope, 65.1; Preston, 54.6; 01–24, 56.2; Dicklow No. 3, 68.5; and Federation, 75.2.

Thus it appears safe to conclude that under the conditions of the experiment, the smut organism had no greater effect on the seedling mortality, on an average, in susceptible than in resistant lines.

DETERMINING THE CLASS INTERVAL

Because of the unknown experimental errors in percentage of infection occurring from single-row plantings, it seemed advisable to make replicate seedings and to determine the size of the errors in the various crosses, and use these results in interpreting the data. The experiments were planned with a view to using Fisher's (*3*) analysis-of-variance method, and the replicates were randomized accordingly. In two of the crosses there was enough seed for three replications, while in the other cross duplicate seedings only were possible. Analysis-of-variance data for the three crosses are given in table 5.

Fisher's (*3*) Z test was made to determine whether or not there was any treatment effect; the value of Z thus calculated is shown at the bottom of the last column of table 5. Since Fisher's (*3*) tables do not give the value for n_1 and n_2 as occurring in the analysis-of-variance tables, it was necessary to calculate this value from the formula given by him. This value is shown at the bottom of the table. The Z quantity, calculated by comparing the variance due to treatment with that due to error, as shown in table 5, is larger in all cases than Z for the 1-percent point. This shows that there is undoubtedly a treatment effect, which naturally was to be expected from the nature of the material, since the mean percentage of smut occurring in the replicated F_3 rows ranged from no smut to a rather high percentage.

TABLE 5.—*Analysis of variance for three wheat crosses*

HOPE × FEDERATION CROSS

Variance due to—	Degrees of freedom	Sum of squares	Mean square	Half log
Replication	1	0.03		
Treatment	206	95,176.77	462.02	3.0678
Error	206	11,459.99	55.63	2.0060
Total	413	106,636.79		Z=1.0584

n_1=206 n_2=206 Z—1-percent point=0.1625

PRESTON × 01-24 CROSS

Replication	2	571.09		
Treatment	306	39,743.41	129.88	2.4335
Error	614	44,335.41	72.44	2.1414
Total	922	116,636.79		Z=.2937

n_1=306 n_2=614 Z—1-percent point=0.1148

HOPE × DICKLOW

Replication	2	149.91		
Treatment	306	78,482.39	256.48	2.7736
Error	614	37,498.94	61.27	2.0579
Total	922	116,131.24		Z=.7157

n_1=306 n_2=614 Z—1-percent point=0.1146

It is evident from table 5 that the variance of a single determination is 55.63 for the Hope × Federation cross, 72.44 for the Preston × 01–24 cross, and 61.27 for the Hope × Dicklow No. 3 cross. These values were used in each case to set up a difference necessary between two mean percentages of infection for a probability of 0.05. Since the error was based on a rather large number, a significant difference would amount to about twice the standard error of the difference. This quantity amounted to about 13 percent in the Hope × Dicklow No. 3 cross, 14 percent in the Preston × 01–24 cross, and 15 percent in the Hope × Federation cross. Then, in classifying the F_3 rows, the value necessary for a significant difference was taken as the class interval; on this basis a frequency table was constructed. The proportion of F_3 rows falling into each of the classes and the reaction of the parental types formed the basis for arriving at the factorial explanation for the inheritance of resistance to loose smut herein suggested.

GENETIC STUDIES OF RESISTANCE TO LOOSE SMUT

The reaction of the parental material to loose smut (*Ustilago tritici*), as shown in table 6, seemed to indicate that possibly more than one factor was involved in the inheritance of resistance. This was also suggested by the breeding behavior of the F_3 progeny of the various crosses. On the basis of these two conditions, it was assumed that at least three factors were involved in the inheritance of resistance to loose smut. This factorial relationship of the parental material is shown in table 6.

Since Hope has never smutted even though thousands of inoculated plants have been grown, it was considered to be completely immune to the inoculum used; therefore, it possessed all three factors in the dominant condition, though dominance is evidently incomplete and the factors have a cumulative effect. Likewise, each of the three factors are thought to have a different effect, an individual with the R_2R_2 factor showing somewhat more resistance than one with the R_3R_3 factor and one with the R_1R_1 factor being about as resistant as one possessing the other two factors. This does not mean that these factors have definite numerical values with specific expression, regardless of the genotype, as factor interaction is not an uncommon phenomenon.

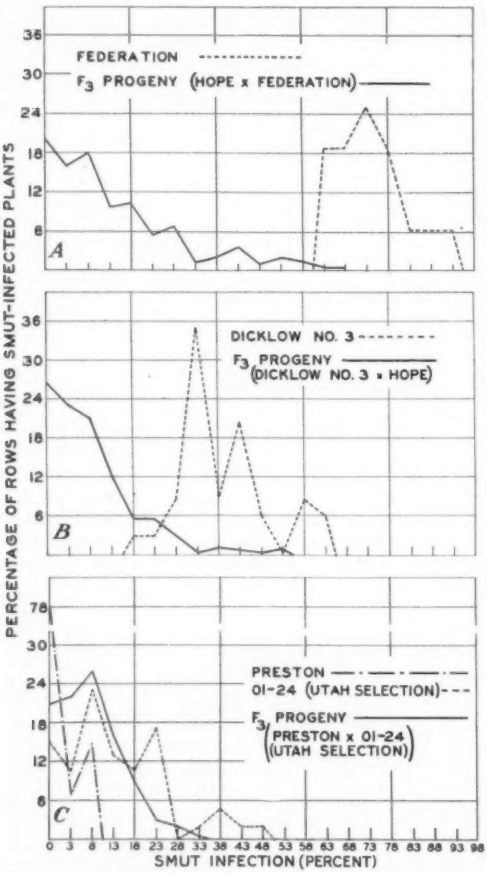

FIGURE 3.—Frequency distribution of parental varieties and F_3 progeny in various wheat crosses as related to loose-smut infection. *A*, Federation and F_3 progeny of Hope × Federation; *B*, Dicklow No. 3 and F_3 progeny of Dicklow No. 3 × Hope; *C*, Preston, 01–24 (Utah selection) and F_3 progeny of Preston × 01–24 (Utah selection).

On an average, Preston has smutted about 1.6 percent, although when it is grown in rows of approximately 30 seeds to the row, similar to the F_3-progeny rows, about 70 percent of the rows show no smut at all; other rows show as much as 9.5 percent smut. Consequently, it was assumed that Preston lacked one of the factors common to Hope and that the absence of this factor allows some smut to develop. Preston would then be classed as highly resistant but not immune, as was Hope.

Under similar conditions, 01–24 smuts about 20 percent. Therefore, it was assumed that it possesses two factors in common with Hope but that it differs from Preston in two factors.

Dicklow No. 3 apparently was more susceptible than 01–24 and, therefore, was assigned only the one factor, R_3R_3; lacking R_1R_1 and

R_2R_2, it is comparatively susceptible and smuts on an average nearly 40 percent.

TABLE 6.—*Genetic composition assigned each of the parental types, on the basis of reaction to loose smut, the range, and the average percentage of infection occurring in rows of 30 kernels each*

Parent	Genotype	Rows	Infection	
			Range	Average
		Number	*Percent*	*Percent*
Hope C.I. 8178	R_1R_1 R_2R_2 R_3R_3	50	0–0	0
Preston C.I. 3081	R_1R_1 R_2R_2 r_3r_3	26	0–9. 5	1. 6±0. 39
01–24 C.I. 11542	r_1r_1 R_2R_2 R_3R_3	47	0–50. 0	18. 5±1. 21
Dicklow No. 3	r_1r_1 r_2r_2 R_3R_3	34	18. 2–61. 9	38. 5±1. 27
Federation	r_1r_1 r_2r_2 r_3r_3	16	60. 9–91. 3	73. 5±1. 57

Federation is assumed to possess none of the factors for resistance, since it ordinarily smuts 70 percent or more.

In table 7 is shown the distribution of the parents and the F_3 of the various crosses in 5-percent classes for loose-smut infection, and figure 3 is a graphical presentation of the same data.

HOPE × FEDERATION CROSS

It is evident from the factorial relationship assigned each parental type that the Hope × Federation cross should give rise to 27 F_2 genotypes. The reaction of the known genotypes, the parents, to the smut inoculum is shown in table 6. On the basis of these known types, the behavior of the remaining genotypes was formulated.

TABLE 7.—*Distribution of parents and F_3 rows of the crosses named in 5-percent classes for loose-smut infection*

Parent or cross	Rows smut-free	Rows having percentages of loose-smut infection falling within the indicated 5-percent classes (average of three replications for F_3; single rows for parents)																				Total rows
		3[a]	8	13	18	23	28	33	38	43	48	53	58	63	68	73	78	83	88	93		
Hope C.I. 8178 number	16																					16
Federation number	0													3	3	4	3	1	1	1		16
91b-Hope (C.I. 8178) × Federation number	43	33	38	20	22	12	13	3	4	7	3	4	3	1	1							207
Hope C.I. 8178 number	34																					34
Dicklow No. 3 (Utah selection) number	0				1	1	3	12	3	7	2	0	3	2								34
87b-Hope C.I. 8178 × Dicklow No. 3 (Utah selection) number	82	71	65	36	17	17	8	3	4	1	0	2										307
Preston C.I. 3081 number	20	2	4																			26
01–24 (Utah selection) number	7	5	11	6	5	8	0	1	2	1	1											47
93b-Preston × 01–24 (Utah selection) number	65	71	81	46	27	9	7	1														306

[a] Taken to the nearest whole number.

The basic phenotypic ratio ordinarily obtained in F_2 in a cross, when three independent factors expressing different characters are involved, is 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1. This ratio, however, may be modified by factor interaction. In the case under consideration the factors were assumed to be of unequal value and were all involved in the expression of a single character. It has been shown that strains even though somewhat susceptible, escape infection when sown in short rows and, therefore, would be placed in the nonsmutting group.

This would tend to make this group too large. To obviate this difficulty, those progenies showing no infection were included in the lowest frequency group. This class interval permitted a certain percentage of infection, which in this cross included those with no infection up to 14.9 percent. The method of arriving at the class interval has previously been discussed. The reason for using the experimental error in arriving at the class interval is that F_3 strains may actually be susceptible up to as much as 15 percent, and yet a certain proportion may escape infection when grown in short rows even though replicated. This would also be true if the interval were extended, but not with the same probability. The phenotypes, the theoretical parental types, and the basis of classification of the F_3 rows in the Hope × Federation cross are shown in table 8.

TABLE 8.—*Possible F_2 genotypes; the theoretical parental type; the percentage of infection, and the basis of classification of the F_3; also the phenotypic ratio in the Hope × Federation cross*

Genotype	Number of each	Theoretical parental type	Actual parental average infection	F_2 classification		
				Class interval	Average infection [a]	Phenotypic ratio
$R_1R_1\ R_2R_2\ R_3R_3$	1	Hope	*Percent* 0	*Percent* 0–14.9	*Percent* 6.9	45
$R_1R_1\ R_2R_2\ R_3r_3$	2					
$R_1R_1\ R_2r_2\ R_3R_3$	2					
$R_1r_1\ R_2R_2\ R_3R_3$	2					
$R_1R_1\ R_2r_2\ R_3r_3$	4					
$R_1r_1\ R_2R_2\ R_3r_3$	4					
$R_1r_1\ R_2r_2\ R_3R_3$	4					
$R_1r_1\ R_2r_2\ R_3r_3$	8					
Total	27					
$R_1R_1\ R_2R_2\ r_3r_3$	1	Preston	1.6			
$R_1R_1\ R_2r_2\ r_3r_3$	2					
$R_1r_1\ R_2R_2\ r_3r_3$	2					
$R_1r_1\ R_2r_2\ r_3r_3$	4					
Total	9					
$R_1R_1\ r_2r_2\ R_3R_3$	1	Intermediate (Preston and 01–24) [b]				
$R_1R_1\ r_2r_2\ R_3r_3$	2					
$R_1r_1\ r_2r_2\ R_3R_3$	2					
$R_1r_1\ r_2r_2\ R_3r_3$	4					
Total	9					
$r_1r_1\ R_2R_2\ R_3R_3$	1	01–24	18.5	15–29.9	19.9	12
$r_1r_1\ R_2R_2\ R_3r_3$	2					
$r_1r_1\ R_2r_2\ R_3R_3$	2					
$r_1r_1\ R_2r_2\ R_3r_3$	4					
Total	9					
$R_1R_1\ r_2r_2\ r_3r_3$	1	Equal to 01–24 [c]	18.5			
$R_1r_1\ r_2r_2\ r_3r_3$	2					
Total	3					
$r_1r_1\ R_2R_2\ r_3r_3$	1	Intermediate (01–24 and Dicklow No. 3). [d]		30–44.9	34.9	3
$r_1r_1\ R_2r_2\ r_3r_3$	2					
Total	3					
$r_1r_1\ r_2r_2\ R_3R_3$	1	Dicklow No. 3	38.5	45–59.9	46.5	3
$r_1r_1\ r_2r_2\ R_3r_3$	2					
Total	3					
$r_1r_1\ r_2r_2\ r_3r_3$	1	Federation	73.5	60–74.9	62.3	1

[a] The average of the class and not the mid point.
[b] Not as resistant as Preston but more resistant than 01–24; somewhere between 1.6 and 18.5 percent.
[c] The R_1R_1 factor was assumed to be equivalent in effect to the R_2R_2 and R_3R_3 factors.
[d] Not as resistant as 01–24 but more resistant than Dicklow No. 3, somewhere between 18.5 and 38.5 percent.

On this basis, Hope, Preston, and the Intermediate (Preston and 01–24) genotypes all fell in the same class. It is evident from table 8 that forty-five sixty-fourths of the F_3 progeny rows would theoretically fall in the first phenotypic group, in which infection would range from 0 to 14.9 percent. In the second group infection ranged from 15 to 29.9 percent; this group included the genotypes corresponding to the 01–24 parental type. Inasmuch as the R_1R_1 factor carried by Hope is assumed to be equal in effect to the other two factors, twelve sixty-fourths of the progeny rows would be expected in the second-class interval. In the third group infection ranged from 30 to 44.9 percent. This group was composed of the genotypes which were neither 01–24 nor Dicklow No. 3 types but which fell somewhere between them; three sixty-fourths of the progeny rows should be of this type. The genotypes corresponding to the Dicklow No. 3 parental type constituted the fourth group, in which infection ranged from 45 to 59.9 percent; three sixty-fourths of the progeny rows would also be expected to be in this class. Infection in the last or upper group ranged from 60 to 74.9 percent; this group included the genotype characteristic of Federation and included only one sixty-fourth of the progeny rows. This completes the phenotypic ratio of 45 : 12 : 3 : 3 : 1. Table 9 shows the goodness of fit obtained when the observed data were fitted to this ratio. The probability is between 0.2 and 0.3 and is considered satisfactory.

TABLE 9.—*Goodness of fit obtained from the breeding behavior in F_3 in regard to loose-smut resistance in the Hope × Federation cross, based on a 45 : 12 : 3 : 3 : 1 ratio*

Smut (percent)	Number of progeny	
	Observed	Calculated
0 to 14.9	132	143.0
15 to 29.9	43	39.4
30 to 44.9	14	9.6
45 to 59.9	13	9.6
60 to 74.9	5	3.2

χ^2_5=5.4085.
P=0.2—0.3.

HOPE × DICKLOW NO. 3

It will be observed that Hope and Dicklow No. 3 differ from each other in two factors and that there will be no genotypes which do not carry the R_3R_3 factor for resistance. The possible F_2 genotypes, the theoretical parental types, the basis of classification of the F_3 rows, and the phenotypic ratio of Hope × Dicklow No. 3 cross are shown in table 10.

TABLE 10.—*Possible F_2 genotypes, the theoretical parental type, percentage infection, and the basis of classification of F_3, also the phenotypic ratio in the Hope × Dicklow No. 3 cross*

Genotype	Number of each	Theoretical parental type	Actual parental average infection	F_3 classification		
				Class interval	Average infection[a]	Phenotypic ratio
$R_1R_1\ R_2R_2\ R_3R_3$	1	Hope	*Percent* 0	*Percent* 0–12.9	*Percent* 6.8	12
$R_1R_1\ R_2r_2\ R_3R_3$	2					
$R_1r_1\ R_2R_2\ R_3R_3$	2					
$R_1r_1\ R_2r_2\ R_3R_3$	4					
Total	9					
$R_1R_1\ r_2r_2\ R_3R_3$	1	Intermediate (Preston and 01–24)[b]				
$R_1r_1\ r_2r_2\ R_3R_3$	2					
Total	3					
$r_1r_1\ R_2R_2\ R_3R_3$	1	01–24	18.5	13–25.9	17.1	3
$r_1r_1\ R_2r_2\ R_3R_3$	2					
Total	3					
$r_1r_1\ r_2r_2\ R_3R_3$	1	Dicklow No. 3	38.5	26–55.0	37.1	1

[a] The average of the class and not the mid point.
[b] Not as resistant as Preston but more resistant than 01–24; somewhere between 1.6 and 18.5 percent.

The amount necessary to give a significant difference between two mean percentage infections, calculated as previously stated (with a probability of 0.05) was approximately 13 percent. Consequently, this was the amount used as the class interval in separating the phenotypic groups and in determining the phenotypic ratio. As in the Hope × Federation cross, no attempt was made to differentiate between the genotypes resembling the Hope parent and those which carried the R_1R_1 and R_3R_3 factors, making them intermediate (Preston and 01–24) types, because they were all included in the same class interval. The range of smut infection allowed in the first phenotypic group was from 0 to 12.9 percent. The class interval in this cross is slightly less than that allowed for the corresponding genotypes of the Hope × Federation cross. This reduction in class interval was due to the slight difference in the variance obtained in this cross and also to the fact that there were triplicate plantings which would reduce the standard error of a difference accordingly. Inasmuch as the class interval is reduced 2 percent in this cross, the range of smut allowed by the various genotypes is correspondingly reduced. Infection in the second phenotypic group ranged from 13 to 25.9 percent and included the genotypes typified by the 01–24 parent. The upper class included all strains with 26 percent infection or more. There were only 20 of the 307 progeny rows that smutted above that amount. This was almost the exact number expected to conform to the Dicklow No. 3 genotype. Infection in the progeny included in this group ranged from 27.1 to 55 percent and averaged 37.05. This is about what would be expected of plants having a genetic make-up equivalent to Dicklow No. 3. For this reason all strains with 26-percent infection or over were considered as one class. This classification gave a phenotypic ratio of 12 : 3 : 1. Table 11 shows the goodness of fit obtained when the observed data were compared with the above ratio. It is evident from table 11 that the goodness of fit was again satisfactory.

TABLE 11.—*Goodness of fit obtained from the breeding behavior in F_3 in regard to loose-smut resistance in the Hope × Dicklow No. 3 cross, based on a 12:3:1 ratio*

Smut (percent)	Number of progeny	
	Observed	Calculated
to 12.9	236	230.2
13 to 25.9	50	57.6
Above 26	20	19.2

$\chi^2 = 1.2169$.
$P = 0.5$–0.7.

In the Preston × 01–24 cross all the factors common to Hope are involved. However, the R_2R_2 factor is present in both parents; as a result, this factor appears in a homozygous dominant condition in all the genotypes obtained from the cross. Data relative to this cross are shown in table 12.

TABLE 12.—*Possible F_2 genotypes, the theoretical parental type, the percentage of infection, and the basis of classification of the F_3, as well as the phenotypic ratio in the Preston × 01–24 cross*

Genotype	Number of each	Theoretical parental type	Actual parental average infection	F_3 classification		
				Class interval	Average infection[a]	Phenotypic ratio
$R_1R_1\ R_2R_2\ R_3R_3$	1	Hope	Percent 0	Percent 0–13.9	Percent 5.8	12
$R_1R_1\ R_2R_2\ R_3r_3$	2					
$R_1r_1\ R_2R_2\ R_3R_3$	2					
$R_1r_1\ R_2R_2\ R_3r_3$	4					
Total	9					
$R_1R_1\ R_2R_2\ r_3r_3$	1	Preston	1.6			
$R_1r_1\ R_2R_2\ r_3r_3$	2					
Total	3					
$r_1r_1\ R_2R_2\ R_3R_3$	1	01–24	18.5	14–27.9	16.9	3
$r_1r_1\ R_2R_2\ R_3r_3$	2					
Total	3					
$r_1r_1\ R_2R_2\ r_3r_3$	1	Intermediate[b] (01–24 and Dicklow No. 3.)		28–41.9	32.0	1

[a] Average of the class and not the mid point.
[b] Not as resistant as 01–24 but more resistant than Dicklow No. 3; somewhere between 18.5 and 38.5 percent.

PRESTON × 01–24

In conformity with the two previously discussed crosses, no distinction was drawn between the genotypes resembling the Hope parent and those resembling the Preston parent, because the range of smut allowed by each came within the range of the class interval. Thus, twelve sixty-fourths of the progeny rows were included in the first phenotypic group. The second group, ranging in infection from 14 to 27.9 percent, was made up of the genotypes resembling the 01–24 parent. Only 10 of the 307 F_3 rows smutted above 28 percent. These ranged in infection from 28 to 39 percent, with an average of 32, which is about as expected since the genotype of this phenotypic

class carries the R_2R_2 factor, which should make it an intermediate (01–24 and Dicklow No. 3) type. From this classification a 12 : 3 : 1 ratio was expected; the goodness of fit obtained when the observed was compared with this ratio is shown in table 13.

TABLE 13.—*Goodness of fit obtained from the breeding behavior in F_3 in regard to loose-smut resistance in the Preston × 01–24 cross based on a 12 : 3 : 1 ratio*

Smut (percent)	Number of progeny	
	Observed	Calculated
0 to 13.9	237	230.2
14 to 27.9	60	57.6
Above 28	10	19.2

$\chi^2=4.7092$.
$P=0.05–0.1$.

It is evident from table 13 that while the fit is not exceptionally good, nevertheless it is within the lower limits of probability usually set (0.05).

FACTORIAL RELATIONSHIP OF THE VARIOUS CROSSES

The fact that the same factors for resistance were assumed to be involved in all three crosses suggests that the progeny of similar genetic constitution obtained from the various crosses ought to give a similar reaction to the inoculum. This relationship is also shown in tables 8, 10, and 12. Infection in the phenotypic class of 45 in the Hope × Federation cross (table 8) ranged from 0 to 14.9 percent, with an average of 6.9; the corresponding class of 12 in the Hope × Dicklow No. 3 cross (table 10) ranged in infection from 0 to 12.9 and averaged 6.7; and the class of 12 in the Preston × 01–24 cross (table 12) ranged in infection from 0 to 13.9, with an average of 5.8. There is a rather close agreement in the mean percentages of infection in the three crosses for the lower class. The Preston × 01–24 cross should show a lower mean infection in the lower class because there were no intermediate (Preston and 01–24) types present. These types appearing in the other two crosses should be more susceptible than the Preston type. It will be noted (table 8, 10, and 12) that the average percentage of infection for the lower phenotypic group in all three crosses is slightly higher than for the Preston parent, which is most typical of the genotypes included in this class. This is to be expected in the Hope × Federation and the Hope × Dicklow No. 3, crosses, however, because the class interval is extended beyond that for Preston, and, therefore, includes intermediate (Preston and 01–24) types, which would be more susceptible than Preston; this would account for the higher average. The average of the lower class in the Preston × 01–24 cross is higher than expected. However, this does not appear especially serious as the same exactness in a study of disease resistance cannot be expected as in a study of morphological characters. This discrepancy may be due to any one or to a combination of the four following conditions:

(1) The heterozygous condition of some of the genotypes, which may allow for more infection than the homozygous, since dominance is not complete.

(2) The effect of modifying factors.

(3) The differential infection in parent and in F_3 progeny due to slight differences in the stage of inoculation. Although instructions were given to inoculate both parent and progeny before or soon after the anthers had shed their pollen (inoculations at later stages appears to reduce materially the amount of infection), it might have been possible that a number of Preston spikes were inoculated somewhat later, when the kernels were partly formed. This would lower the average percentage of infection of Preston. In this connection it might be well to mention that in 1933 Preston showed 9.5 percent of infection when inoculated with the same smut. It is not known whether this was due to differences in stages of inoculation or to soil and climatic differences.

(4) It is possible that one of the parents, especially Preston, was a mixed population. Therefore, the pure line of Preston used in this cross may have been slightly more susceptible than the average for the variety. This would necessitate a slight change in the genetic constitution of Preston from $R_1R_1R_2R_2r_3r_3$ to one of a type $R_1R_1r_2r_2$ R_3R_3 since the R_3R_3 factor allows more infection than the R_2R_2 factor. One would now expect the theoretical average of the first class to be considerably above 1.6 percent.

In view of these various possibilities, it appears desirable to leave the genetic composition of the varieties as shown in table 6.

The average percentage of infection for genotypes similar to 01–24 and Dicklow No. 3, in all crosses was within a few percent of these parental types (tables 8, 10, and 12). In the Hope × Federation cross (table 8) the 45 progeny observed in the upper class had an average of infection lower than that of the Federation parent, although they were within the range of the Federation; with the few progeny rows the differences may be due purely to error in sampling. In the Hope × Dicklow No. 3 cross no types were recovered with any higher range of infection than that of the Dicklow No. 3 parent. The 20 rows falling in this class had an average infection of 37.1 as compared with 38.5 for Dicklow No. 3. The Preston × 01–24 cross gave no genotypes which did not carry the R_2R_2 factor; hence, the highest infection expected was somewhere between the range of the parental varieties 01–24 and Dicklow No. 3. This would be somewhere between 18.6 and 38.5 percent. The average actually obtained for this class was 32 percent (table 12). In general, it appears as though the genotypes recovered from the various crosses corresponding to the parental types react to loose smut similarly to the parental types used and as though the similar genotypes recurring in the different crosses behave in a similar manner.

STUDIES ON THE INHERITANCE OF MORPHOLOGICAL CHARACTERS AND THEIR RELATION TO RESISTANCE TO LOOSE SMUT

Inheritance of Morphological Characters

The primary purpose in obtaining the breeding behavior of the awns, chaff, and kernel color was to determine whether or not there was any relationship between any of these morphological characters and disease resistance. The knowledge of such a relationship, if it did exist, would be of great value to the plant breeder.

AWNS

HOPE × DICKLOW NO. 3 AND PRESTON × 01–24 CROSSES.—The awn inheritance of Hope × Dicklow No. 3 and of Preston × 01–24

FIGURE 4.—Inheritance of awns in the Hope × Dicklow No. 3 and Preston 01-24 crosses: *A*, Short, apica awns typical of Dicklow No. 3 and 01-24; *B*, short, apical awns of the F_1; *C*, fully awned spikes common to Hope and to Preston; *D*, *E*, *F*, awn classes found in F_2.

crosses was similar. In both crosses one of the parents was fully awned, while the other parent had short, apical awns. The awns of the F_1 plants were of intermediate length, although they resembled

more closely the short-awned parent. Both parental types and a group of intermediates were recovered in the F_2 of each cross. The intermediates segregated in F_3; thus three distinct awn classes were

FIGURE 5.—Inheritance of awns in the Hope × Federation cross: *A*, Federation; *B*, F_1; *C*, Hope; *D*, *E*, *F*, *G*, awn classes found in F_2.

obtained: (1) Short, apical awns, such as those in the Dicklow No. 3, and the 01–24 parents; (2) segregating types; and (3) fully awned specimens like the Hope and Preston parents. The parental, F_1 and F_2 types are shown in figure 4. Table 14 shows the observed

and calculated numbers falling in each class and the goodness of fit based on a 1:2:1 ratio. It is apparent from table 14 that a good fit was obtained in both crosses.

TABLE 14.—*F_3 breeding behavior of awns in the Hope × Dicklow No. 3 and the Preston × 01–24 crosses, and the goodness of fit based on a 1:2:1 ratio*

Parent [a]	Class	Observed	Calculated	χ^2	P
Hope (*AABB*) × Dicklow No. 3 (*aaBB*).	Short apical awns	82	76.7		0.7–0.8
	Segregating	150	153.3	0.4890	
	Fully awned	75	76.7		
Preston (*AABB*) × 01–24 (*aaBB*).	Short apical awns	78	76.7		
	Segregating	158	153.4	.5835	.7–.8
	Fully awned	71	76.7		

[a] Awnedness was arbitrarily chosen to be represented by the dominant characters; awnlessness might just as appropriately be so designated.

HOPE × FEDERATION CROSS.—The inheritance of awns in the Hope × Federation cross was quite different from that in the Hope × Dicklow No. 3 and Preston × 01–24 crosses just discussed. The F_1 in the Hope × Federation cross was intermediate in inheritance but again resembled most closely the awnless parent. The parental types, the F_1, and the true-breeding F_3 types of the Hope × Federation cross are shown in figure 5. Besides the four homozygous types shown, there were five segregating classes of progeny: (1) Those segregating for awn classes 1 and 2; (2) those segregating for awn classes 1,2, and 3; (3) those segregating for awn classes 1, 2, 3, and 4; (4) those segregating for awn classes 2, 3, and 4; and (5) those segregating for awn classes 3 and 4. There were, therefore, nine genotypic classes into which the F_3 progenies were classified. This breeding behavior suggested a two-factor difference with independent segregation. The relation of the observed to the calculated based on a two-factor difference and the closeness of fit to a 1:2:2:4:1:2:1:2:1 ratio is shown in table 15. It is evident from this table that a good fit was obtained.

TABLE 15.—*Breeding behavior of awns in the Hope × Federation cross and the goodness of fit to a 1:2:2:4:1:2:1:2:1 ratio*

F_3 breeding behavior	Observed	Calculated	χ^2	P
True breeding 4	15	13.0		
Segregating 3, 4	20	26.0		
Segregating 2, 3, 4	25	26.0		
Segregating 1, 2, 3, 4	50	52.0		
True breeding 3	16	13.0	3.3077	0.90–0.95
Segregating 1, 2, 3	25	26.0		
True breeding 2	14	13.0		
Segregating 1, 2	26	26.0		
True breeding 1	16	13.0		

KERNEL COLOR

Kernel-color inheritance was involved in all three crosses. The F_1 plants all had red grain and segregation took place in F_2.

HOPE × FEDERATION AND HOPE × DICKLOW NO. 3 CROSSES.—The proportion of white to red kernels in the F_2 in the Hope × Federation and Hope × Dicklow No. 3 suggested a three-factor difference, with each factor either alone or in combination expressing the character.

In the Hope × Dicklow No. 3 cross 5 plants out of 307 had white grain; it was assumed to be similar to the Hope × Federation cross, which had 4 white-kerneled plants out of 206 F_2's. Therefore, the Hope × Dicklow No. 3 cross was not studied for grain color in the F_3.

Studies made on the inheritance of kernel color in F_3 in the Hope × Federation cross behaved as would be expected from a study of the F_2 data. With a three-factor difference, the F_3 should theoretically segregate into 5 classes, giving a 37 : 12 : 8 : 6 : 1 ratio. The five classes into which the F_3's were classified, based on kernel color, are shown in table 16. In this table is also shown the observed number in each class which were fitted to a 37 : 12 : 8 : 6 : 1 ratio with a good fit resulting, as shown by the χ^2 test.

TABLE 16.—*Breeding behavior for grain color in the Hope × Federation cross and the goodness of fit based on a 37 : 12 : 8 : 6 : 1 ratio*

Class	Number of progeny		χ^2	P
	Observed	Calculated		
True-breeding red	122	118.4	0.7941	0.9–0.95
Segregating 15:1	36	38.4		
Segregating 63:1	23	25.6		
Segregating 3:1	21	19.2		
True-breeding white	4	3.2		

PRESTON × 01–24 CROSS.—Kernel-color studies in the F_2 generation in the Preston × 01–24 cross suggested a single-factor difference. The segregation in F_3 substantiated the results of the findings in the previous generation. Three classes were observed in F_3. The proportion of the F_3 rows falling in each of these classes is shown in table 17. The χ^2 test shows a good fit to the expected 1 : 2 : 1 ratio.

TABLE 17.—*Breeding behavior for grain color in the Preston × 01–24 cross and the goodness of fit based on a 1 : 2 : 1 ratio*

Class	Number of progeny		χ^2	P
	Observed	Calculated		
True-breeding white	82	76.7	0.5039	0.7–0.8
Segregating	149	153.4		
True-breeding red	76	76.7		

GLUME COLOR

HOPE × FEDERATION AND PRESTON × 01–24.—The F_2 data on the two crosses, Hope × Federation and Preston × 01–24, indicated that in each case there was a single-factor difference for chaff color. In the Hope × Dicklow No. 3 cross both parents had white chaff, and thus no segregation occurred. Table 18 shows the F_3 breeding behavior of the two crosses for chaff color and the goodness of fit based on a 1 : 2 : 1 ratio. The χ^2 test shows a good fit in both crosses.

TABLE 18.—*Breeding behavior for glume color in the Hope × Federation and Preston × 01–24 crosses and the goodness of fit based on a 1 : 2 : 1 ratio*

Parent	Class	Number of progeny		χ^2	P
		Observed	Calculated		
Hope × Federation	True-breeding white	56	51.7		
	Segregating	99	103.4	0.7032	0.7–0.8
	True-breeding bronze	52	51.7		
Preston × 01–24	True-breeding white	76	76.7		
	Segregating	149	153.4	.4988	.7–0.8
	True-breeding bronze	82	76.7		

RELATION OF MORPHOLOGICAL CHARACTERS AND RESISTANCE TO LOOSE SMUT

In order to determine whether a relationship exists between morphological characters and resistance to loose smut, a series of contingency tables was prepared showing a comparison of the reaction to smut infection and morphological characters. A measure of the relationship between the distribution of the two characters being compared may be obtained by calculating χ^2 and determining the value of P from Fisher's (*3*) tables. Table 19 shows the distributions and the probability obtained in each case. Table 20 gives a summary of all comparisons. In interpreting results it is safe to assume that if the value of P for any given distribution is higher than 0.05, there is no evidence of significant correlation between the characters being considered.

TABLE 19.—*Contingency table for grain color, chaff color, and awns and smut classes as occurring in the F_3 progeny of various crosses*

GRAIN COLOR AND SMUT CLASSES IN PRESTON × 01-24 CROSS

Smut (percent)	Number of progeny				χ^2	P
	White	Segregating	Red	Total		
0 to 13.9	59	114	64	237		
14 to 27.9	15	29	16	60		
28 to 41	2	6	2	10	0.5489	0.2–0.3
Total	76	149	82	307		

GRAIN COLOR AND SMUT CLASSES IN THE HOPE × FEDERATION CROSS

0 to 14.9	1	51	82	134		
15 to 29.9	2	15	24	41		
30 to 44.9	1	7	6	14		
45 to 59.9	0	5	8	13	6.4055	0.5–0.7
60 to 74.9	0	2	3	5		
Total	4	80	123	207		

CHAFF COLOR AND SMUT CLASSES IN THE PRESTON × 01-24 CROSS

Smut (percent)	Number of progeny				χ^2	P
	White	Segregating	Bronze	Total		
0 to 13.9	61	116	60	237		
14 to 27.9	13	27	20	60	2.1727	0.7–0.8
28 to 41.9	2	6	2	10		
Total	76	149	82	307		

TABLE 19.—*Contingency table for grain color, chaff color, and awns and smut classes as occurring in the F_2 progeny of various crosses*—Continued

CHAFF COLOR AND SMUT CLASSES IN THE HOPE × FEDERATION CROSS

Smut (percent)	Number of progeny				χ^2	P
	White	Segregating	Bronze	Total		
0 to 14.9	38	65	29	132		
15 to 29.9	9	24	10	43		
30 to 44.9	6	4	4	14		
45 to 59.9	2	5	6	13		
60 to 74.9	1	1	3	5		
Total	56	99	52	207	10.8819	0.2–0.3

AWNS AND SMUT CLASSES IN THE PRESTON × 01–24 CROSS

Smut (percent)	Number of progeny				χ^2	P
	No. 1	Segregating	No. 4	Total		
0 to 13.9	62	123	52	237		
14 to 27.9	14	29	17	60		
28 to 41.9	2	6	2	10		
Total	78	158	71	307	1.4196	0.8–0.9

AWNS AND SMUT CLASSES IN THE HOPE × DICKLOW NO. 3 CROSS

0 to 12.9	64	113	59	236		
13 to 25.9	13	26	11	50		
26 to 55.0	5	11	5	21		
Total	82	150	75	307	0.4486	0.95–0.98

AWNS AND SMUT CLASSES IN THE HOPE × FEDERATION CROSS

Smut (percent)	Number of progeny					χ^2	P
	No. 1.	No. 2	No. 3	No. 4	Total		
0 to 14.9	7	10	9	10	36		
15 to 29.9	4	2	2	3	11		
30 to 44.9	3	1	3	1	8		
45 to 59.9	1	1	2	1	5		
60 to 74.9	1	0	0	0	1		
Total	16	14	16	15	61	6.9854	0.8–0.9

Since the lowest P value in table 20 is between 0.2 and 0.3, there seems to be no indication of relationship between resistance or susceptibility and any of the morphological characters studied. This is interesting in view of the fact that Fromme (*5*, *6*) has reported that awned varieties were more susceptible to loose smut than were awnless varieties. The question arises, was there any relationship between these two characters from the standpoint of inheritance, or did it just happen that those awned varieties with which Fromme was dealing smutted more than the awnless? The studies herein reported indicate that there is no relationship between awns and susceptibility or resistance; in fact, the awned varieties used in these studies were resistant, and the awnless were susceptible.

TABLE 20.—*Summary of the x^2, and P values as given in table 19*

Cross	Characters tested for relationship	x^2	P
Hope × Federation	Grain color and smut reaction	6.4055	0.5 –0.7
Preston × 01–24	do	.5489	.2 – .3
Preston × 01–24	Chaff color and smut reaction	2.1727	.7 – .8
Hope × Federation	do	10.8819	.2 – .3
Preston × 01–24	Awns and smut reaction	1.4196	.8 – .9
Hope × Dicklow No. 3	do	.4486	.95– .98
Hope × Federation	do	6.9854	.8 – .9

SUMMARY

Genetic studies on the inheritance of loose-smut resistance, awns, grain color, and chaff color are reported for the Hope × Federation, Hope × Dicklow No. 3, and Preston × 01–24 crosses.

The relative effects of the time and method of inoculation as related to infection are also reported. Maximum infection was obtained only when the smut spores were placed directly on the stigmas. There appeared to be little or no difference in the amount of infection occurring when the plants were inoculated at the time the stamens were rather green and immature and when they were inoculated when the plants were in bloom. In the inheritance studies on loose smut, triplicate randomized plantings were made in two of the crosses and duplicate seedings in the other. This permitted a statistical study of the size of the experimental error. The analysis-of-variance method was used in these studies from the error obtained in the various crosses, it was used to determine a difference which might be considered as significant, on the basis of a probablility of 0.05 between two percentages of infection. The amount obtained was used as the class interval in determining the inheritance of resistance to loose smut. The number of F_3 rows falling within the various class intervals, along with the reaction of the parental material to the disease, formed the basis for the proposed factorial relationship.

The genetic studies of awns and both kernel and chaff color were made in the usual way. The proposed genetic composition of the parental material, for the characters studied, based on their behavior in the previously mentioned crosses is given in table 21.

There was no evidence in the studies made of any relationship between the morphological characters and resistance to loose smut.

TABLE 21.—*Proposed genetic composition of parental material for characters studied in crosses made*

Variety	Loose smut	Awns	Kernel color	Glume color
Hope	$R_1R_1\ R_2R_2\ R_3R_3$	*AA BB*	$K_1K_1\ K_2K_2\ K_3K_3$	*gg*
Preston	$R_1R_1\ R_2R_2\ r_3r_3$	*AA BB*	$K_1K_1\ k_2k_2\ k_3k_3$	*gg*
01–24	$r_1r_1\ R_2R_2\ R_3R_3$	*aa BB*	$k_1k_1\ k_2k_2\ k_3k_3$	*GG*
Dicklow No. 3	$r_1r_1\ r_2r_2\ R_3R_3$	*aa BB*	$k_1k_1\ k_2k_2\ k_3k_3$	*gg*
Federation	$r_1r_1\ r_2r_2\ r_3r_3$	*aa bb*	$k_1k_1\ k_2k_2\ k_3k_3$	*GG*

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THE MANGANESE CONTENT OF GRASSES AND ALFALFA FROM GRAZED PLOTS [1]

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INTRODUCTION

The nutritive value of manganese and its occurrence in animal and plant tissue has received considerable attention within the last few years. The literature relating to manganese in plant tissue has been reviewed by Lindow and Peterson (*6*)[3], who reported analyses of 84 samples representing the principal classes of human foods. In a later paper by Skinner and Peterson (*12*), analyses of 54 feeding stuffs were given, which show a wide variation in the manganese content of plant material. Several other investigators (*9, pp. 25–26*) have contributed to the knowledge of the occurrence of manganese in foods and feeding stuffs.

In this paper is shown the manganese content of orchard grass (*Dactylis glomerata* L.), domestic rye (*Secale cereale* L.), tall oatgrass (*Arrhenatherum elatius* Beauv.), meadow fescue (*Festuca elatior* L.), timothy (*Phleum pratense* L.), Kentucky bluegrass (*Poa pratensis* L.), redtop (*Agrostis palustris* Huds.), bromegrass (*Bromus inermis*), and alfalfa (*Medicago sativa* L.). The samples used for analysis were collected from the experimental substation plots at Caldwell, Idaho. Just before the plots were grazed, samples from each were taken and sent to the laboratory for analysis. The samples were then dried, ashed, and analyzed as described in the following paragraphs.

METHODS OF ANALYSIS

Since the anhydrous sodium carbonate fusion of the ash in biological material had shown increased recovery of calcium and magnesium (*3, 8*), it was felt that the method might well be applied to the colorimetric determination of manganese. The simplicity of the method and its many advantages in manipulation recommended it as a rapid and accurate means of getting complete recovery of manganese in plant tissue.

One to two grams of the air-dry material to be analyzed was weighed into a platinum dish and ashed overnight in an electric muffle at 600° C. The ash was then fused with 3 g of anhydrous sodium carbonate. After cooling, the fused mass was placed in a 250-cc beaker and covered with a watch glass. Distilled water was added in sufficient quantity to moisten the sample and then 15 cc of 20-percent sulphuric acid (by volume) was added. When the fused material was dissolved, it was washed from the platinum crucible with

[1] Received for publication Dec. 6, 1933; issued June 1934. Research Paper No. 111 of the Idaho Agricultural Experiment Station. Presented before the eighty sixth meeting of the American Chemical Society at Chicago, Ill., September 1933.

[2] The author wishes to express his appreciation to Prof. Harry P. Magnuson for providing the opportunity for this study and for many helpful suggestions in the preparation of the paper. It is desired, also, to express appreciation to Reuben F. Johnson, superintendent of the Caldwell substation, for the collection of the grass samples.

[3] Reference is made by number (italic) to Literature Cited, p. 662.

a few cubic centimeters of 5-percent sulphuric acid. A few drops of 15-percent sodium bisulphite were added to the solution until all the manganese was reduced to manganous sulphate. The solution was then boiled to expel excess sulphur dioxide, filtered, and the acid-insoluble residue washed with several small portions of 5-percent sulphuric acid.

To the filtrate was added approximately 0.3 g of potassium periodate. The beaker was covered with a watch glass and its contents boiled for 5 minutes. The solution was allowed to stand for 1 hour at a temperature of 95° to 100° C. to insure complete oxidation of the manganese. The solution was then diluted to 90 to 95 cc with 5-percent sulphuric acid previously boiled with a little potassium periodate, and cooled to room temperature, the entire sample transferred to a 100-cc colorimetric tube and compared in a colorimeter with a standard manganese solution. A standard solution containing 0.0025 mg of manganese per cubic centimeter was a satisfactory one to use.

The chlorides were not removed from the samples; this step seemed unnecessary because of the small amount that is present in plant material. According to Willard and Greathouse (*14*), the presence of chlorides does not interfere with the final development of color, as the chlorides may be driven off by prolonging the time of oxidation and adding an excess of potassium periodate. Richards (*11*) has shown that excess acidity prevents full development of color or causes it to fade. The acidity, therefore, was kept between 5 and 6 percent. In the large number of samples analyzed no fading of color was observed. It was possible to let the samples stand for several hours and check the first reading, provided they were kept free from the fumes in the laboratory.

The marked agreement in the results of analyses of 1-g samples of the same grass (orchard grass), is shown in the following tabulation:

Sample no.—	*Manganese (milligrams per kilogram)*
1	177. 7
2	177. 7
3	176. 5
4	181. 7
5	178. 7
6	180. 0
7	179. 2
8	179. 2
Average	178. 8

As a further test of the method, known quantities of manganese were added to samples of air-dry timothy hay, and recovery determinations were then made. Table 1 shows that excellent recovery was obtained, considering the fact that a very small quantity of manganese was added as compared with that present in the sample. The difference in recovery of manganese was well within the experimental error of the colorimeter.

TABLE 1.—*Recovery of known quantities of manganese added as manganese sulphate to 2 grams of air-dry timothy hay*

Manganese added (milligram)	Total manganese found	Manganese in grass taken	Added manganese found	Recovery	Manganese added (milligram)	Total manganese found	Manganese in grass taken	Added manganese found	Recovery
	Milligram	*Milligram*	*Milligram*	*Percent*		*Milligram*	*Milligram*	*Milligram*	*Percent*
	0.210	0.1825	0.0275	110		0.210	0.1825	0.0275	92
0.025	.207	.1825	.0245	98	0.030	.212	.1825	.0295	98
	.206	.1825	.0235	94		.215	.1825	.0325	108
	.232	.1825	.0495	99		.205	.1825	.0225	112
0.050	.230	.1825	.0475	95	0.020	.200	.1825	.0175	87
	.235	.1825	.0525	105		.203	.1825	.0205	102
	.197	.1825	.0145	97		.215	.1825	.0325	93
0.015	.195	.1825	.0125	83	0.035	.218	.1825	.0355	101
	.195	.1825	.0125	83		.220	.1825	.0375	107

A comparison of the percentage recovery of manganese by the sodium carbonate fusion method, by the hydrofluoric-sulphuric acid digestion method (*1*), and by the official method (*5*) is presented in table 2. The hydrofluoric-sulphuric acid digestion method was used as a base of 100-percent recovery to facilitate the comparison. It will be observed that the results obtained by the anhydrous sodium carbonate fusion method showed very good agreement with those obtained by the hydrofluoric-sulphuric acid digestion method, but the recovery of manganese ranged from 15 to 35 percent lower by the official method than by the other two methods. The sodium carbonate fusion method has been used in a large number of analyses of plant material and has given very satisfactory results.

TABLE 2.—*Comparison of the 3 different methods used for the analysis of manganese in 7 grasses*

Grass	Hydrofluoric-sulfuric acid digestion of plant ash	Sodium carbonate fusion of plant ash	Official method	Sodium carbonate fusion	Official method
				Hydrofluoric acid digestion	Hydrofluoric acid digestion
	Mg per kg	*Mg per kg*	*Mg per kg*	*Percent*	*Percent*
Orchard grass	150	150	122	100.0	81.3
Domestic rye	122	120	102	98.3	83.6
Tall oatgrass	110	111	94	100.9	85.4
Meadow fescue	105	107	90	101.9	85.7
Kentucky bluegrass	77	76	64	98.7	83.1
Bromegrass	150	152	110	101.3	73.3
Redtop	230	228	150	99.1	65.2

EXPERIMENTAL PLOTS

The soil used for the experimental plots is a Boise sandy loam. It is described (*4, p. 426*) as a

> . . . . grayish-colored light sandy loam, with a soft, ashy feel, carrying a large amount of silt and having an average depth of about 2 feet. The subsoil of this type south of Boise River is a loam or clay loam which has an average depth of about 18 to 24 inches. This in turn is underlain usually with a sandy loam, but sometimes with sand, generally cemented together with calcium carbonate, forming a hardpan.

In the late summer of 1930 one area of a field was fenced and divided into 9 plots. These plots were about 25 feet wide and 20 rods long, the length of the plots being parallel to the slope of the field. All the plots had uniform drainage, slope, and irrigation. Prior to the experimental work the following crops had been grown on this soil: In 1921, corn; in 1922 and 1923, barley; in 1924 and 1925, wheat; and in 1926 to 1930, alfalfa.

The plots were grazed according to good pasture practice. Every 2 weeks during the grazing season dairy heifers were allowed to graze on the plots for 1 to 3 days. After the stock had been removed, the plots were irrigated, but no fertilizer was applied to any of them during the experiment.

Since the plots were grazed instead of clipped, definite yield data were not obtained. However, the approximate height of each variety of grass was determined at the time the sample was taken for analysis, that is, just before the heifers were admitted to the plots every 2 weeks throughout the grazing season. The fastest growing grass, tall oatgrass, averaged 6.4 inches in height; orchard grass, 3.6 inches; bromegrass, meadow fescue, redtop, timothy, and domestic rye, averaged 2.6 inches. Kentucky bluegrass, the slowest growing grass, averaged 1.9 inches. Alfalfa averaged 5.5 inches. All the grasses, and the alfalfa, grew more rapidly in the early part of the season.

A representative sample of soil from each of the plots was taken during the grazing season and analyzed for manganese and calcium carbonate, and the pH values were determined. The manganese was determined as described in the official methods (*7*). Calcium carbonate was determined by the Puri method (*10*) and the pH values by the hydrogen-electrode method. The soils of all the plots were uniform in manganese and calcium carbonate and in pH values (table 3).

TABLE 3.—*pH and percentage of manganese and calcium carbonate of the soil of 9 pasture plots*

Plot planted in—	Manganese	Calcium carbonate	pH	Plot planted in—	Manganese	Calcium carbonate	pH
	Percent	*Percent*			*Percent*	*Percent*	
Orchard grass	0.0706	1.00	7.86	Bromegrass	0.0715	0.75	7.90
Domestic rye	.0710	.75	7.23	Redtop	.0712	.75	7.86
Tall oatgrass	.0735	.75	7.85	Alfalfa	.0712	.75	7.72
Meadow fescue	.0703	.75	7.65				
Timothy	.0711	.75	7.55	Average	.07123	.777	7.72
Kentucky bluegrass	.0707	.75	7.86				

RESULTS OF ANALYSIS

Table 4 presents the analyses of samples of orchard grass, domestic rye, tall oatgrass, meadow fescue, timothy, Kentucky bluegrass, bromegrass, redtop, and alfalfa, taken throughout the grazing season.

TABLE 4.—*Manganese content (milligram per kilogram of grass on oven-dry basis) of 8 varieties of grasses and 1 alfalfa, grown under grazing conditions*

Date taken	Orchard grass	Domestic rye	Tall oatgrass	Meadow fescue	Timothy	Kentucky bluegrass	Brome grass	Redtop	Alfalfa
1932									
June 6	268	197	105	174	86	69	172	188	61
June 20	240	130	81	127	86	77	172	161	41
July 4	292	145	91	160	97	70	166	161	39
July 18	240	120	90	149	97	75	172	214	49
Aug. 1	214	85	90	154	106	107	134	214	40
Aug. 15	170	97	82	184	150	83	134	161	47
Aug. 29	214	97	105	176	130	73	134	177	47
Sept. 11	158	90	121	154	130	75	107	161	47
Sept. 26	165	123	126	110	142	80	152	244	48
Oct. 11	114	135	113	124	120	72	203	240	47
Average	207.5	121.9	100.4	151.2	114.4	78.1	154.6	192.1	46.6

Wide variations are shown in the manganese content of samples of a single variety of grass taken on different dates. In spite of this, certain grasses showed a relatively higher manganese content than others. Orchard grass had the highest average manganese content of all the grasses. Next in order came redtop, bromegrass, meadow fescue, domestic rye, timothy, tall oatgrass, and Kentucky bluegrass.

Alfalfa had a lower manganese content than any of the grasses. Since comparative yield data were lacking, it was impossible to compare the total amount of manganese recovered by alfalfa with the total amount recovered by the grasses. A rapid-growing and high-yielding species like alfalfa, although low in manganese, would show a relatively high total recovery of manganese for the season.

Since the soils of the nine plots showed a uniform manganese content, and since all the plots had received the same treatment, it would seem that the difference in manganese content in the eight varieties of grasses was not due to the difference in manganese content of the soil or to a difference in soil conditions. Moreover, no correlation was found between the rate of growth of the eight grasses and their manganese content, nor between the rate of growth of a single variety of grass and its manganese content. It was concluded, therefore, that the difference in manganese content in the eight grasses was due to the difference in the capacity of the grasses for extracting manganese from the soil.

As a check on the foregoing conclusion the writer calculated, from the field data of Wiggans (*13*), the total amount of manganese recovered by seven of the grasses used in this experiment. Wiggans gives the average yield over a 4-year period of several grasses cut as pasture. His yield data, compiled by Ellenberger, Newlander, and Jones (*2, p. 6*), give the yield in pounds of dry matter per acre, as follows: Timothy, 1,454 pounds; redtop, 1,557 pounds; meadow fescue, 1,665 pounds; orchard grass, 1,711 pounds; Kentucky bluegrass, 1,387 pounds; bromegrass, 1,690 pounds; tall oatgrass, 2,044 pounds.

The total amount of manganese recovered for each grass in pounds per acre, based upon the preceding yield data, was as follows: Orchard grass, 0.0355 pound; redtop, 0.0299 pound; bromegrass, 0.0262 pound; meadow fescue, 0.0252 pound; tall oatgrass, 0.0205 pound; timothy, 0.0166 pound; and Kentucky blue grass, 0.0109 pound. With the

exception of tall oatgrass, there was a close correlation between the total amount of manganese recovered by the grasses during the grazing season and their manganese content. The exception of tall oatgrass was to be expected, as this was one of the fastest growing and largest yielding grasses.

Since complete information on the manganese requirements of animals, and on the effect of an excess or deficiency of manganese in grasses is not available, it is impossible to determine to what extent the feeding value of the pasture is affected by its manganese content. It is clearly shown from the data presented, however, that the manganese content of pasture may be increased by growing such grasses as orchard grass, redtop, bromegrass, and meadow fescue.

SUMMARY

A new method for the determination of manganese in plant material by the fusion of the plant ash with anhydrous sodium carbonate is presented. This method gives a greater recovery of manganese than is obtained by the official methods.

The manganese content of eight grasses is shown. The average manganese content (dry basis) ranged from 207.5 mg per kilogram for orchard grass to 78.1 mg per kilogram for Kentucky bluegrass. Alfalfa, with an average of 46.6 mg per kilogram was lower in manganese than any of the grasses.

The eight grasses varied markedly in their capacity to extract manganese from the soil.

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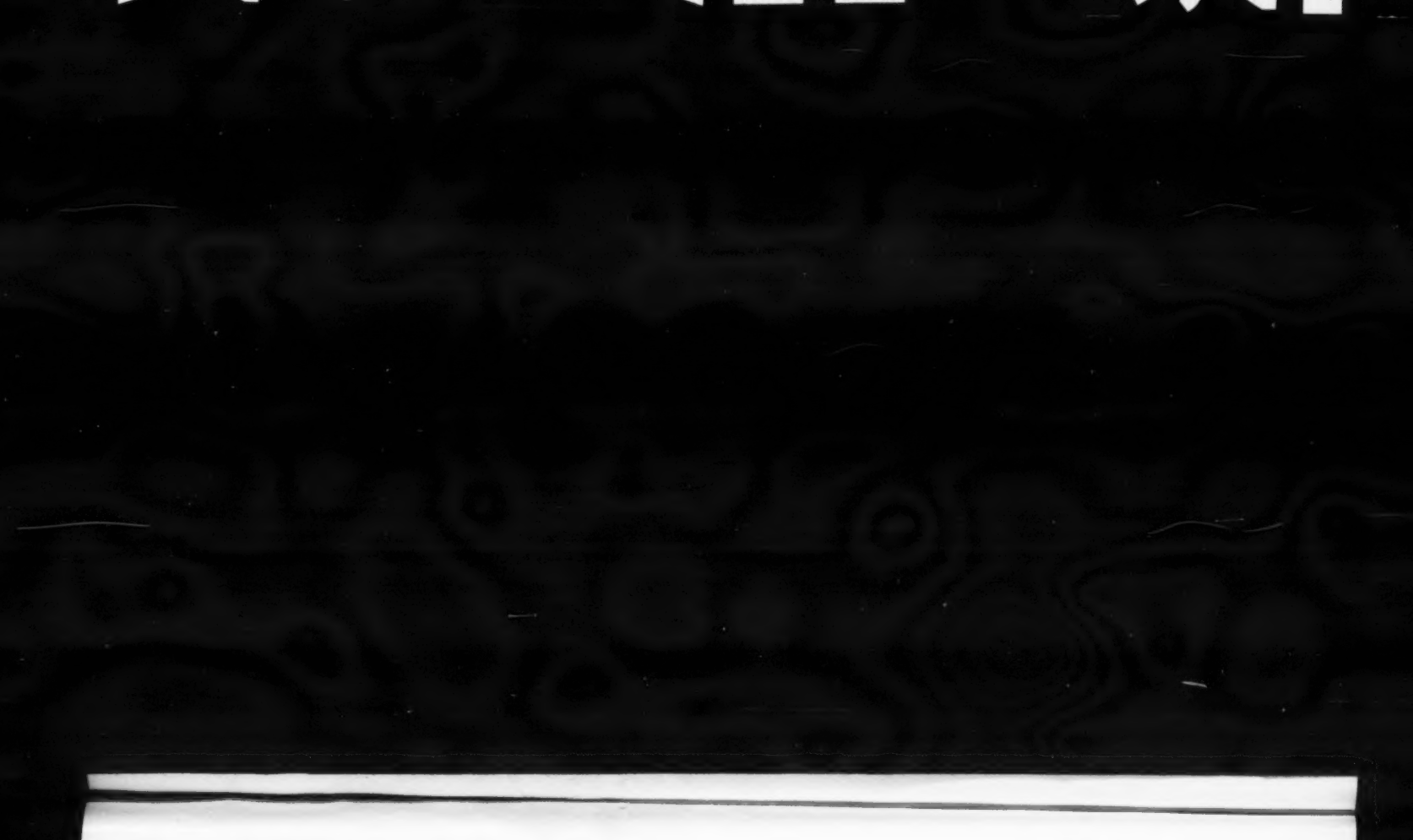
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